

KINETICS-EFFECT RELATIONS OF GLIPIZIDE

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Abstract An HPLC method has been developed that allows selective and sensitive measurements of glipizide and other sulfonylureas in plasma, and this method has been used to study kinetics-effect relations of glipizide in subjects with type 2 diabetes and in healthy volunteers. It appears that glipizide has a rapid absorption, complete bioavailability, a small volume of distribution (10 l) and a short elimination half-life (below 5 h), which is maintained during long-term treatment. Glipizide has a potent blood glucose lowering capacity, being effective already at doses of 1 mg, and at plasma concentrations above 50-100 nmol/l. Its effect disappears at the same time as the drug has been eliminated from blood. Glipizide may undergo some enterohepatic recirculation, and this may help to explain why glipizide is particularly efficient on non-fasting glucose levels. The effect of glipizide can be improved if the drug is given 1/2 h before breakfast, as this enhances its absorption rate and promotes a more appropriate temporal relation between the augmentation of insulin release and the metabolic impact of the meal. Daily doses above 15 mg seem to give little or no therapeutic profit and may even lead to impairment instead. The major effect of glipizide is to augment insulin availability in response to meals, whilst it has little influence on nocturnal glucose control. At a daily dose of 7.5 mg or more, glipizide can be taken once daily without loss of efficacy, at least in areas with Northern European meal schedules		
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Edvard W. W. W. W.

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- I. E. Wåhlin-Boll and A. Melander. High-performance liquid chromatographic determination of glipizide and some other sulfonylurea drugs in serum. *Journal of Chromatography* 164:541-546, 1979.
- II. E. Wåhlin-Boll, L.-O. Almér and A. Melander. Bioavailability, pharmacokinetics and effects of glipizide in type 2 diabetics. *Clinical Pharmacokinetics* 7:363-372, 1982.
- III. E. Wåhlin-Boll, G. Sartor, P.-O. Bitzén, M. Brune and A. Melander. Steady-state elimination rate and enterohepatic recirculation of glipizide. *Clinical Pharmacokinetics* (submitted).
- IV. E. Wåhlin-Boll, A. Melander, G. Sartor and B. Scherstén. Influence of food intake on the absorption and effect of glipizide in diabetics and in healthy subjects. *European Journal of Clinical Pharmacology* 18:279-283, 1980.
- V. E. Wåhlin-Boll, G. Sartor, A. Melander and B. Scherstén. Impaired effect of sulfonylurea following increased dosage. *European Journal of Clinical Pharmacology* 22:21-25, 1982.
- VI. E. Wåhlin-Boll, L. Groop, S. Kahumaa, P.-H. Groop, K.-J. Tötterman and A. Melander. Therapeutic equivalence of once- and thrice-daily glipizide. *European Journal of Clinical Pharmacology* (in press).

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INTRODUCTION

Diabetes in primary health care

In Sweden, primary health care has a defined responsibility for the health of the population. This gives particular emphasis to preventive and early therapeutic measures in major chronic diseases. Among these is diabetes mellitus, which in Scandinavia has an overall prevalence of about 2% (1) and a prevalence of about 10% in subjects above the age of 60 (2).

Characteristics and definitions of diabetes

The pathognomonic metabolic characteristic of diabetes mellitus is hyperglycemia, and it is likely, although not ascertained, that chronic hyperglycemia is the main cause of the long-term complications of the disease, involving blood vessels and nerves.

Diabetes mellitus is commonly divided in two different categories; the so-called insulin-dependent form, or type 1, characterized by an absolute insulin deficiency, and the so-called non-insulin-dependent form, or type 2, characterized by a reduced insulin sensitivity and a relative insulin deficiency.

Type 2 diabetes and its management

Type 2 diabetes mellitus comprises more than 75% of all cases of diabetes (1). It usually affects individuals above the age of 50 and was previously referred to as maturity-onset diabetes. Its appearance and development is slower and less dramatic than that of type 1, and a reasonable degree of metabolic control can often be achieved without pharmacologic intervention. Additional reduction of type 2 hyperglycemia can be obtained by drugs administered orally. The drugs used most frequently are the sulfonylureas.

Sulfonylureas

The first indication that oral agents could reduce blood glucose came in 1942 when it was discovered that an antibacterial sulfonamide evoked hypoglycemia as an adverse effect (3). Subsequently, another sulfonamide, carbutamide, was developed. This sulfonamide, which is a sulfonylurea (SU), had a pronounced blood-glucose lowering capacity but retained the antibacterial effect (4). In addition, its toxicity was considerable. Soon thereafter tolbutamide, a less toxic SU lacking the antibacterial moiety, was introduced, and it reached worldwide acceptance in the management of type 2 diabetes. During the 1960's a number of other SU compounds became used in clinical practice e.g. acetohexamide, tolazamide and chlorpropamide. Like tolbutamide, these compounds are given in gram doses and the corresponding serum levels are in the range of μmol - mmol/l (5). During the 1970's more potent SU have been introduced clinically, namely, glibenclamide and glipizide. They are given in milligram doses and the corresponding serum levels are in the nmol - $\mu\text{mol/l}$ range (6, 7). The two "second-generation" SU nowadays dominate the SU market in Sweden and several other countries.

Mechanism of action of SU

It is not yet settled whether all SU have the same mechanism(s) of action. Unquestionably, all SU enhance insulin release from the B cells (8), and at least glipizide can increase insulin availability by reducing the hepatic extraction of the hormone (9, 10). It is widely held that SU may improve insulin sensitivity in peripheral tissues (11, 12), but it is possible that this is secondary to the increased availability of insulin (13). It remains unclear if SU interfere with the release of other pancreatic hormones. Even if all SU have the same mechanism(s) of action, they may nevertheless differ in their clinical efficacy and toxicity, because of differences in potency and pharmacokinetics.

Glipizide

As the aim of diabetes treatment should be complete normalisation of glucose economy, the ideal SU should be as potent as possible. It should also be rapid-acting in order to promote maximum reduction of the glucose increase following meals. The effect duration is important from both the efficacy and the safety point of view; it should be long enough to maintain euglycemia over a 24-hour period, but also short enough to avoid long-lasting hypoglycemia. In addition, the ideal SU should have rapid absorption and complete bioavailability, in order to minimize pharmacokinetic variations between patients.

At the time of its clinical introduction, it was evident that glipizide was a potent, rapid- and short-acting SU and would hence meet some of the above-mentioned criteria. Little was known, however, about its bioavailability and pharmacokinetics, as well as about its kinetics-effect relations and its appropriate dosage.

AIMS

The aims of the present investigation were:

- 1) to develop a selective, sensitive and reproducible method to measure glipizide concentrations in human serum
- 2) to measure and describe the bioavailability and kinetics of glipizide in patients with type 2 diabetes
- 3) to assess how glipizide kinetics relates to the therapeutic effect of the drug
- 4) to establish the appropriate dosage range and dose interval.

SUBJECTS AND METHODS

Diabetic patients

Forty male and 26 female patients with type 2 diabetes were studied. They were all being treated with conventional diet regulation. In addition, some patients were on sulfonylurea medication (papers III, V and VI). None had any signs of major diabetic complications, no history of recent myocardial infarction nor clinical evidence of cardiac, pulmonary, hepatic or renal dysfunction (except for one patient in paper III with hepatic cirrhosis). Details are given in the separate papers.

Healthy volunteers

Fifteen young healthy male volunteers participated in the studies described in papers III and IV, where also details concerning age and weight are given.

Measurements of glipizide

The development of the method used to measure serum glipizide in papers II-VI is described in paper I. Lately, this method has been modified regarding extraction media (dichloromethane/hexane has replaced benzene) and analytical column (a 5 μm particle column has replaced the former 10 μm particle column). The use of a new detector, a Waters Absorbance Detector Model 441 wavelength set at 229 nm, has greatly diminished the baseline "noise", thereby making chromatogram interpretation easier. This modified version of the method was used for drug determinations in paper III.

Measurement of chlorpropamide

Serum chlorpropamide (paper V) was analyzed by gas chromatography.

Blood glucose

Venous blood was used in the determination of blood glucose by conventional enzymatic methods (papers II-VI).

Urine glucose

The 24-hour urine glucose excretion (paper VI) was determined by polarimetry.

HbA₁

Glycosylated haemoglobins (HbA₁) (paper VI) were determined by microcolumn chromatography.

Plasma or serum insulin

Commercially available radioimmunoassay kits were used for the determination of plasma or serum insulin (papers IV-VI).

Serum C-peptide

The serum concentrations of C-peptide were determined by a radioimmunoassay (paper VI).

Pharmacokinetic calculations

In paper I, calculations were carried out according to Wagner (14). A two compartment open model where elimination occurs only from compartment no. 1 seemed to fit the drug concentration curves following 1 mg glipizide given intravenously. The following equations were used:

$$k_{21} = \frac{C_o \times \beta + B \times \alpha}{C_o + B}$$

$$\alpha \times \beta = k_{21} \times k_{el}$$

$$\alpha + \beta = k_{12} + k_{21} + k_{el}$$

$$V_1 = \frac{D}{C_0}$$

$$V_{d \text{ area}} = \frac{D}{\beta \times AUC_{0-\infty}}$$

$$Cl = \frac{D}{AUC_{0-\infty}}$$

$$\text{residual area} = \frac{C_{pt}}{\beta}$$

Abbreviations

C_0 = concentration of drug in compartment 1 at time 0 given by extrapolation of the α -phase to time 0

B = concentration of drug in compartment 1 at time 0 given by extrapolation of the β -phase to time 0

D = dose

α = elimination rate constant during the α -phase calculated after feathering and $\alpha = \frac{\ln 2}{t_{\frac{1}{2}}}$ (for the α -phase)

β = elimination rate constant during the β -phase $= \frac{\ln 2}{t_{\frac{1}{2}}}$ (for the β -phase)

k_{21} = first order rate constant for transfer of drug from compartment no. 2 to compartment no. 1

k_{el} = first order rate constant for elimination of drug by all processes from compartment no. 1

k_{12} = first order rate constant for transfer of drug from compartment no. 1 to compartment no. 2

V_1 = volume of compartment no. 1

Cl = mean plasma clearance

AUC = area under the concentration curve estimated by the trapezoidal rule

C_{pt} = the drug concentration at the last sampling time

$t_{\frac{1}{2}}$ = drug elimination half-life were calculated by regression analysis

$C_{\max}$ = maximum concentration recorded

$t_{\max}$ = time to reach $C_{\max}$

Statistics

The statistical significance of differences was calculated by the Wilcoxon signed rank test (papers II, V and VI) or Student's paired t-test (paper IV).

RESULTS AND DISCUSSION

Development of an analytical method for selective and sensitive determination of glipizide in human serum (paper I)

Pharmacokinetic studies necessitate assay methods that are sensitive and sufficiently selective. Most drugs are extensively metabolized; hence the method must be able to measure the drug in the presence of structurally very similar compounds. Drugs are biotransformed in order to produce metabolites polar enough to be eliminated through the kidneys. High pressure liquid chromatography with reversed phase columns elute substances according to polarity, and this is the reason why it is especially well suited for drug analyses in serum and other body fluids. Apart from the drug and its possible metabolites, serum contains numerous endogenous substances, such as proteins and lipids, which enforce a serum sample clean-up in order to isolate and concentrate the drug and also to protect the analytical system from getting clogged up. This can usually be achieved by extraction of the drug at an optimal pH from the aqueous serum sample into an organic phase, followed by evaporation.

As glipizide is a weak acid with a pK_a of 5.94 (15), the pH in serum as well as in the mobile phase was set at 3.5. The yield of glipizide into the most

suitable extraction medium, benzene, was 84%. A mobile phase consisting of 40% 0.001 M phosphate buffer and 60% methanol in combination with a reversed phase column allowed the rather unpolar glipizide as well as the very unpolar internal standard, glibornuride, to elute at a sufficient distance after co-extracted serum compounds. Glipizide is metabolized in the liver to at least two hydroxylated metabolites. They are both more polar than glipizide itself and were hence eluted in the serum front. The detection limit was 20 nmol/l, the intra-assay variation 1.8% and the inter-assay variation 6.2% (both tested at the 200 nmol/l level). The method was also found suitable for the determination of other SU, namely, chlorpropamide, tolbutamide, glibenclamide and glibornuride.

Recently dichloromethane/hexane 50/50 has replaced benzene as extraction medium due to the environmental hazards of the latter. The yield into this mixture is somewhat less than with benzene but still suitable when glipizide concentrations are above 100 nmol/l. When glipizide concentrations were lower, a mixture of 30% hexane in dichloromethane was found to be more appropriate. However, this mixture locates the serum on top which makes the transfer of the extraction medium into the evaporation tubes more laborious and time-consuming; hence the 50/50 mixture is preferred whenever possible.

An analytical column containing Spherisorb-ODS 5 μ m particles (Brown Lee HPLC system) instead of the former 10 μ m ones has increased resolution and shortened the elution time, and has hence been used when possible. The detector system has been changed to a Waters Absorbance Detector Model 441 used at 229 nm. This has resulted in a more stable baseline with a minimized "noise" level, making the interpretation of the chromatogram easier. The overall reproducibility of the method is excellent and there is no serious interference from plasma extracts.

Bioavailability, pharmacokinetics and effects of single doses of glipizide in patients with type 2 diabetes (paper II)

Adequate pharmacotherapy includes not only adequate drug selection but also appropriate dosage and administration of drug(s) selected. This necessitates both pharmacodynamic and pharmacokinetic studies in man, preferably in patients with the disease in question. Six elderly patients with type 2 diabetes participated in this study. They were examined on five different occasions: 1) without any glipizide administration, and following 2) 1 mg glipizide i.v., 3) 2.5 mg glipizide solution p.o., 4) 2.5 mg glipizide tablet p.o., and 5) 5 mg glipizide tablet p.o. The drug was always ingested on an empty stomach. Subsequently, the patients were served standardized meals for breakfast, lunch and dinner. The tests were carried out with at least one day in between.

Blood samples were taken at regular intervals for analyses of blood glucose and serum glipizide concentrations.

The oral bioavailability of glipizide, i.e. the dose-corrected ratio $\frac{\text{AUC p.o.}}{\text{AUC i.v.}}$, was very close to 100 per cent with each oral dose. This is consistent with the assumption that glipizide is completely absorbed, and that there is negligible presystemic biotransformation of the drug. In some cases, glipizide bioavailability exceeded 100 per cent, suggesting enterohepatic recirculation of the drug (see further below and paper III).

The absorption of 2.5 mg glipizide from the solution was very rapid, the peak concentration being reached within half an hour. When the same dose was administered as a tablet, the peak concentration was lower and the time to reach peak concentration longer, suggesting a slower absorption due to the extra time needed for tablet disintegration and glipizide dissolution.

The serum concentration curve following 1 mg glipizide given i.v. suggested that glipizide pharmacokinetics can be described by a two-compartment model. There was no indication of an additional deep compartment following this dose.

The volume of distribution was small, for the central compartment 4.4 l and V_d area 10.2 l, which is typical of an acidic drug highly bound to plasma albumin. The apparent elimination half-life (the half-life of the β -phase) following i.v. administration of 1 mg glipizide and after oral intake of 2.5 mg glipizide in solution was about 2.5 h. The elimination half-life of glipizide was longer following oral intake of the drug in tablet form, the mean value being 3-3.5 h.* This might be explained by prolonged absorption from the tablets, so that the elimination phase was misjudged. Alternatively, it cannot be excluded that glipizide is distributed to some deep (third) compartment, (the elimination from which does not become apparent until a certain dose level is reached). A further possibility is that glipizide undergoes entero-hepatic recirculation; this is also suggested by the fact that its bioavailability sometimes exceeded 100 per cent (see above). In additional support of this possibility, one patient displayed distinct second peaks following ingestion of the lunch meal (see also paper III). This was particularly striking when the drug had been taken in tablets.

Each dose of glipizide reduced the total of blood glucose AUC in 5 of the 6 patients. Patient no. 6 contracted common cold during the study, which probably explains why she had an increasing fasting blood glucose. However, the net area of blood glucose was found to decrease following glipizide. Accordingly, it can be claimed that each dose of glipizide was effective in each patient.

* In the original article there are three printing errors in Table II: the $t_{1/2}$ of subject 4 AG under b should be 2.3 h, not 2 m 3 h, that of subject 5^{1/2} JA under b should be 4.2 h, not 2.8 h, and under d should be 5.0 h not 3.0 h.

The effect of glipizide on blood glucose seemed to last for about the same time as the drug was present in blood, i.e. 4 h for the 1 mg dose and 6-12 h for the 2.5 - 5 mg doses. This goes well along with the fact that glipizide has a small volume of distribution and a short elimination half-life, implying that most of the drug is present in blood and that little or no drug would be remaining in the tissues when the drug has been eliminated from blood. These findings also argue against the view that glipizide would be distributed to a slowly equilibrating deep compartment, although this cannot be completely excluded in a single-dose study.

To summarize, glipizide appears to have rapid absorption, complete bioavailability, a small volume of distribution and a short elimination half-life (below 5 h). It has a potent blood glucose lowering capacity, being effective already at the dose of 1 mg, and at plasma concentrations above 50-100 nmol/l. Its effect disappears at the same time as the drug has been eliminated from blood. It is possible that glipizide undergoes some enterohepatic recirculation and, even though it seems unlikely, these single-dose data cannot entirely exclude that glipizide is partially distributed to a slowly equilibrating deep compartment. The latter aspects are dealt with in paper III.

Steady-state elimination rate and enterohepatic recirculation of glipizide (paper III)

The preceding single-dose investigation (paper II) showed that the plasma elimination half-life of glipizide is less than 5 h, and that the effect duration equals the time of drug presence in plasma. The latter fits well with the fact that glipizide is a lipophilic, weakly acidic drug with a high degree of binding to plasma albumin (15) and (hence) a very small apparent volume of distribution (10 l) (paper II). It may seem surprising therefore, that treatment with once-daily administration of glipizide can be as effective as thrice-daily intake (16). This has also been verified in paper VI. This fostered

the question whether the elimination of glipizide would be prolonged during steady-state conditions, and it is noteworthy that a study employing radio-labelled glipizide suggested the existence of a slowly equilibrating deep compartment of the drug (17). Another possibility is that glipizide undergoes enterohepatic recirculation, an idea supported by the fact that the apparent oral bioavailability of glipizide sometimes exceeds 100 per cent (paper II). In addition, secondary peaks in the time-concentration curves have been observed following meals (paper II; 18). Meal-induced recirculation of glipizide could also help to explain why this drug is particularly efficient in reducing blood glucose increases following meals (paper VI).

In order to assess whether the plasma elimination half-life could be prolonged during chronic administration, serum samples for glipizide determinations were taken during a mean of 16.5 hours from 12 patients with type 2 diabetes who had been treated with glipizide for a minimum of one month. The elimination half-life of glipizide in these 12 patients ranged between 2.38 and 4.63 hours. This signifies that a sufficiently long period (corresponding to a mean of 5.1 half-lives) had elapsed to allow the conclusions that the terminal elimination half-life of glipizide is not longer than 5 hours and that there is no accumulation or saturation process involved in the handling of glipizide.

As indicated by two previous studies (paper II; 18), and re-emphasized in the current one, the elimination pattern of plasma glipizide is altered shortly after lunch intake, with the appearance of secondary peaks, suggesting enterohepatic recirculation of the drug. A very high secondary peak, exceeding the primary peak, was seen at lunch time in a patient with hepatic cirrhosis. She even displayed a third peak at dinner time. This could be explained by a reduced hepatic capacity to metabolise glipizide, with more drug being excreted in the bile.

In order to assess this possibility further, the influence of exogenous cholecystokinin on the single-dose kinetics of glipizide was studied in 6 healthy volunteers. Five of the 6 volunteers showed either a second peak in their serum glipizide time-concentration curves or a transient slowdown in glipizide elimination shortly after the cholecystokinin injection. Concurrently, minor reductions in blood glucose levels could be recorded. Accordingly, it seems reasonable to assume that glipizide may undergo enterohepatic recirculation to a minor degree, and this may help to explain why once-daily administration of glipizide may be therapeutically equal to thrice-daily intake, and why glipizide is particularly efficient on non-fasting blood glucose levels (see paper VI).

Influence of food intake on the absorption and effect of glipizide in healthy subjects and in patients with type 2 diabetes (paper IV)

Adequate diet regulation is a major function in the control of diabetes. It must be important, therefore, to establish the most appropriate relations between the intake of food and the intake of glipizide. This leads to the question whether food intake would influence the rate and extent of glipizide absorption and, if so, whether this influence would affect blood glucose levels. These aspects are dealt with in paper IV, which comprises single-dose studies in 9 healthy volunteers and in 14 patients with type 2 diabetes. Neither had been exposed to SU previously. The former took the drug together with (at the end of) breakfast and on an empty stomach (fasting continued for 4 h), while the latter took the drug together with (at the end of) breakfast and 30 min before breakfast.

In the volunteers, glipizide evoked an increase in plasma insulin and reduced blood glucose levels both during continued fasting and when taken with the meal. Food intake did not affect the peak concentration, the elimination half-life or the bioavailability of the drug, but the absorption of glipizide

was delayed by about 1/2 h. As food intake reduces the rate of gastric emptying, and as glipizide dissolves rapidly in (simulated) enteric fluid but slowly and poorly in (simulated) gastric fluid (19), it is assumed that the delay is an expression of delayed gastric emptying and hence of glipizide dissolution.

In the patients, administration of glipizide 1/2 h before breakfast augmented plasma insulin levels to the same extent as intake together with breakfast but the former administration caused a much greater blood glucose reduction. This was reasonably due to the earlier appearance of glipizide in blood and the earlier increase in insulin secretion, promoting a more appropriate temporal relation between the augmentation of insulin release and the metabolic impact of the breakfast meal. These single-dose data suggest that the therapeutic response would be augmented by administration 1/2 h before breakfast, although they do not prove that also the chronic therapeutic effect would be improved by systematic intake of glipizide before meals.

Impaired effect of glipizide following increased dosage (paper V)

Even though single oral doses of glipizide have pronounced blood glucose reducing effects in patients with type 2 diabetes (papers II-IV) this need not signify that long-term treatment with glipizide will be successful. At least for other SU, there is vast clinical experience that long-term SU treatment fails to maintain a sufficient reduction of blood glucose. This may be due to a true long-term inefficiency of SU, but it is also possible that inadequate dosage may contribute to the therapeutic failure.

In order to examine how different dosages might influence the therapeutic efficacy of glipizide, 10 patients with type 2 diabetes were examined during long-term treatment with this drug at two different dosage levels during monitoring of the plasma concentrations of the drug. In addition, a cross-over comparison was made with chlorpropamide treatment.

For at least one month, 5 of the patients were initially kept on chlorpropamide and the other 5 on glipizide. The former 5 were then switched to glipizide, and vice versa. After an additional month on the second treatment, its dosage was increased. After at least another month on the third treatment the patients on high dosage chlorpropamide were switched to high dosage glipizide and vice versa. The resulting mean daily doses were 14.75 ($5.25 + 4.75 + 4.75$) and 25 ($8.5 + 8.0 + 8.5$) mg for glipizide, and 278 and 431 mg once daily for chlorpropamide. The drugs were always ingested before meals.

Neither the fasting plasma insulin nor the fasting blood glucose levels differed significantly between treatments, and there was no significant change in body weight. Hence it was assumed that the different responses in relation to meals were due to differences in the drug treatments rather than to variations in disease intensity and/or in compliance with dietary regulations. In addition, it was assumed that there was adequate compliance with drug therapy, as the dosage increases were accompanied by doubling of the mean drug levels. Furthermore, it was seen that, with one exception for each drug, all patients were continuously exposed to SU already at the lower dosage. Moreover, as even the exceptional cases became continuously exposed after the increase in dose, there was no particular reason to suspect that these subjects had complied less well with their medication than the others.

In spite of the marked elevation in plasma chlorpropamide concentration after the dosage increase, neither plasma insulin nor blood glucose responses showed any improvement. It seems likely, therefore, that the maximum effect of chlorpropamide had been reached already at the lower dosage. Alternatively, the concentration-effect curve for chlorpropamide may be so flat that doubling of the drug concentration would not suffice to enhance the therapeutic effect of the drug. The lower dosage of glipizide promoted a better therapeutic effect than either dose of chlorpropamide, as judged from

the improvement in blood glucose levels. This emphasizes the higher potency of the second generation SU glipizide and suggests that an improved blood glucose control can be achieved by a switch over from chlorpropamide to glipizide. As there was no corresponding increase in plasma insulin levels, the improved glucose utilization may have resulted from an extrapancreatic effect. However, as C-peptide levels were not determined in this study, it cannot be excluded that the improvement was secondary to enhanced insulin release from the pancreas. Moreover, the finding of unchanged insulin levels in the presence of blood glucose reduction does not necessarily indicate a primary increase in insulin sensitivity; such an effect could be secondary to the preceding increase of insulin secretion and the subsequent reduction in blood glucose (13).

Although the lower dosage of glipizide improved blood glucose control, the increase in glipizide dosage to 25 mg did not further improve therapeutic efficacy. On the contrary, the blood glucose concentrations were significantly elevated and sometimes even exceeded the levels recorded during chlorpropamide treatment. Such impairment was seen both in those who were on high-dosage glipizide beforehand and in those who took it after chlorpropamide. Therefore, the impaired effect could hardly have been due to progression of the disease, but was rather a consequence of treatment. Furthermore, the impairment could not be explained by reduced availability of insulin, as the plasma insulin levels were higher rather than lower after the dosage increase.

The impaired glucose utilization seen during high-dosage glipizide could be secondary to effects on the secretion of other hormones e.g. glucagon, but it could also be secondary to increased insulin resistance subsequent to increased insulin exposure.

In conclusion, glipizide may offer a therapeutic advantage over chlorprop-

amide but the therapeutic effect may be restricted not only by limitations set by the disease but also by counter-regulatory mechanisms that develop during continuous exposure to high levels of glipizide. It appears that dosage beyond 15 mg daily gives little or no therapeutic profit and may even lead to impairment instead.

Therapeutic equivalence of once- and thrice-daily glipizide (paper VI)

The short half-life of glipizide would seem to justify administration in three or more daily doses and, as the efficacy of glipizide is increased when the drug is taken half an hour before breakfast (paper IV), it is common practice to administer glipizide before the three major meals. On the other hand, it has been reported that the degree of glucose control was similar when glipizide (7.5-20 mg) was given once daily before breakfast and when given before breakfast, lunch and early (5 p.m.) dinner (17). However, this does not preclude that later administration of the third dose would improve nocturnal and fasting glucose levels. Paper VI examined this possibility by cross-over comparisons of once daily administration with two different thrice daily schedules, the third dose given before early (5 p.m.) dinner (study 1, n=11) and at 10 p.m. (study 2, n=12), respectively. The dosage was either 7.5 or 15 mg daily, and therapy was maintained for 2 x 8 weeks (study 1) and 2 x 4 weeks (study 2).

Neither the 24-hour urinary glucose excretion nor HbA_{1c}, fasting plasma glucose, insulin or C-peptide levels differed between once-daily and thrice-daily administration with the third dose given before early dinner. The nadir levels of glipizide were not significantly different and were often too low to be detected. As expected, the after-breakfast levels of glipizide were higher during once-daily dosage, and there were corresponding improvements in plasma insulin and glucose. Postponing the third dose until 10 p.m. led to considerably higher nocturnal/fasting levels of glipizide than during once-daily

treatment but this was not associated with any improvement in HbA_{1c} , or in fasting plasma glucose, insulin or C-peptide.

These findings support the conclusion that the major effect of glipizide is to augment insulin availability following meals, whilst it has little influence on nocturnal glucose control. This might in part relate to meal-induced recirculation of glipizide (paper III). At a daily dose of 7.5 mg or more, glipizide can be taken once daily without loss of efficacy, at least in areas with Northern European meal schedules.

GENERAL SUMMARY AND CONCLUSIONS

An HPLC method has been developed that allows selective and sensitive measurements of glipizide and other sulfonylureas in plasma, and this method has been used to study kinetics-effect relations of glipizide in subjects with type 2 diabetes and in healthy volunteers.

It appears that glipizide has a rapid absorption, complete bioavailability, a small volume of distribution (10 l) and a short elimination half-life (below 5 h), which is maintained during long-term treatment. Glipizide has a potent blood glucose lowering capacity, being effective already at doses of 1 mg, and at plasma concentrations above 50-100 nmol/l. Its effect disappears at the same time as the drug has been eliminated from blood. Glipizide may undergo some enterohepatic recirculation, and this may help to explain why glipizide is particularly efficient on non-fasting glucose levels.

The effect of glipizide can be improved if the drug is given 1/2 h before breakfast, as this enhances its absorption rate and promotes a more appropriate temporal relation between the augmentation of insulin release and the metabolic impact of the meal. Daily doses above 15 mg seem to give little

or no therapeutic profit and may even lead to impairment instead. The major effect of glipizide is to augment insulin availability in response to meals, whilst it has little influence on nocturnal glucose control. At a daily dose of 7.5 mg or more, glipizide can be taken once daily without loss of efficacy, at least in areas with Northern European meal schedules.

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Biomedical Applications

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Note

High-performance liquid chromatographic determination of glipizide and some other sulfonylurea drugs in serum

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(Received May 16th, 1979)

Sulfonylurea drugs are widely used in the treatment of diabetes mellitus of the maturity-onset character. The first sulfonylurea generation, comprising compounds such as tolbutamide and chlorpropamide, is being gradually replaced by second-generation sulfonylureas, such as glibenclamide and glipizide, which offer a markedly enhanced potency without a corresponding increase in toxicity. Sensitive and selective chemical methods for the determination of blood concentrations of tolbutamide and chlorpropamide are available and have been applied in clinical studies [1–3]. For the second-generation drugs, however, no such methods have been developed so far.

The present report describes a high-performance liquid-chromatographic (HPLC) technique for the measurement of glipizide concentrations in blood. The method also permits the determination of the blood concentrations of tolbutamide, chlorpropamide and glibenclamide. Glibornuride is used as internal standard in the assay of glipizide and glibenclamide. Chlorpropamide serves as internal standard for tolbutamide and vice versa.

MATERIALS AND METHODS

The technique described below is the finally adopted method for the measurement of glipizide concentrations in serum. The influence of variations in extraction media, pH, internal standards, and UV absorption wavelengths are described under Results and discussion, and so are the techniques for measurement of glibenclamide, tolbutamide and chlorpropamide.

Apparatus

A Waters Model 6000 pump, equipped with a U6K injector and a Varian Vari-Chrom UV–Vis detector were used.

Column

μ Bondapak C₁₈ columns (0.3 m $\times$ 3.9 mm I.D.; 10- μ m particles) were obtained prepacked from Waters Associates, Göteborg, Sweden.

Chemicals

Mixtures of methanol and phosphate buffers were used for elution. Degassing was carried out by sonication for 15 min. All reagents employed were of analytical grade and were used without further purification. Glipizide (Mindiab[®]) and two glipizide metabolites, the 3-cishydroxycyclohexyl and the 4-transhydroxycyclohexyl derivatives were kindly supplied by Dr. Tosolini, Istituto Carlo Erba per Ricerche Terapeutiche, Milan, Italy. Glibenclamide (Euglucon[®]) and tolbutamide (Artosin[®]) were gifts from Dr. V. Hrtska, Boehringer-Mannheim, Mannheim, G.F.R. Glibornuride (Glutril[®], Hoffman-LaRoche & Co., Basle, Switzerland) was used as internal standard. Chlorpropamide (Diabinese[®]) was obtained from Pfizer Corp., Groton, Conn., U.S.A. Standard solutions were made in methanol and were found to be stable for at least 3 months when kept refrigerated.

Extraction procedure

Eight hundred nanograms of glibornuride (internal standard) and 1 ml of HCl (0.05 mol/l) were added to 0.5 ml of serum, resulting in a pH of about 3. Extraction was made with 3 ml of benzene, during gentle automated shaking for 10 min. After centrifugation, the organic phase was transferred to a conical tube and evaporated to dryness in a water-bath at 45° under a stream of air. The extract was re-dissolved in 50 μ l of methanol. An aliquot of 20 μ l was injected into the chromatograph.

Blood samples

Venous blood samples were obtained from patients on medication with glipizide, glibenclamide, tolbutamide or chlorpropamide. Serum was prepared by centrifugation, and was stored at -20° until assayed. For the initial method development, and for making serum standards, known amounts of the different drugs were added to drug-free serum.

RESULTS AND DISCUSSION

Extraction media

The following extraction media were tried: hexane, diethyl ether, toluene, chloroform, benzene, methylene chloride, ethylene dichloride, butanol, and hexane-diethyl ether (1:1). Of these, only benzene was found suitable, as the others either extracted glipizide badly or yielded interfering peaks from their dried residues.

Influence of pH on glipizide yield

Glipizide is a weak acid with a pK_a of 5.94 [4]. As it was the aim to keep the drug undissociated to the largest possible degree, a pH of 3.0–3.5 seemed appropriate for the extraction. It was found that the yield was maintained at about 84% up to pH 5. At pH 6 and 7, the yield had fallen to 4%.

Influence of phosphate buffer on glipizide capacity factor

Changes in the amount of phosphate buffer in the mobile phase altered the capacity factor for glipizide as shown in Fig. 1. It appeared that a content of 40% phosphate buffer of 0.01 mol/l and a pH of 3.5 would be optimal.

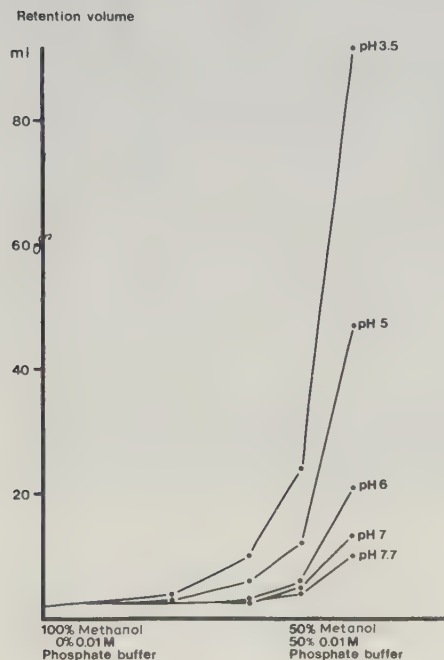


Fig. 1. Influence on the capacity factor for glipizide of amount of phosphate buffer and of pH in the mobile phase.

Detection wavelength and limit

Absorption maxima were 225 nm for glipizide and 275 nm for glibornuride. The molar absorption at the chosen detection wavelength, 225 nm, was found to be 24,000 for glipizide and 16,000 for glibornuride. The detection limit for glipizide was 10 ng/ml, using an injection volume of 20 μ l. The sensitivity of the spectrophotometer was kept on 0.02 a.u.f.s. throughout. A typical chromatogram is shown in Fig. 2.

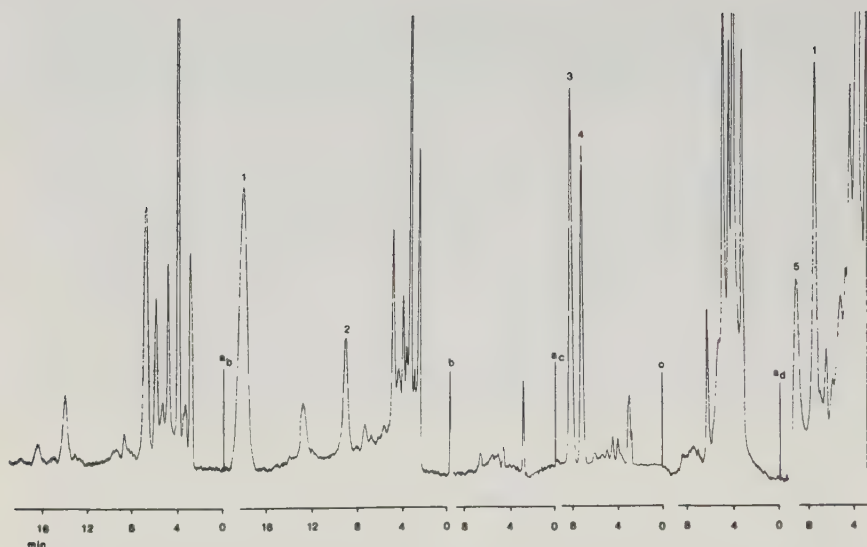


Fig. 2. Chromatograms of serum blanks (a_b , a_c , a_d), and serum from patients being treated with glipizide (b), chlorpropamide (c) and glibenclamide (d). Peaks: 1 = glibornuride, 2 = glipizide, 3 = tolbutamide, 4 = chlorpropamide, 5 = glibenclamide.

Quantitation

The standard curve was linear over a range of 20–1500 ng of glipizide. Analyses of the same sample ten times in the same run yielded a standard deviation of 1.8%. Inter-assay was found to be 6.2% (S.D.) as judged from eleven consecutive assays.

Internal standards

The following compounds were tested as internal standards: tolbutamide, chlorpropamide, glibenclamide, methylglipizide and glibornuride. Of these, only glibornuride and glibenclamide had a retention volume that was not equal to that of any endogenous compound. Efforts to clean up serum were unsuccessful. Under conditions optimal for glipizide detection, both glibornuride and glibenclamide eluted later than glipizide. Glibornuride eluted closer to glipizide than did glibenclamide and was hence chosen as internal standard. The total retention volume was 18 ml.

Metabolites

Neither of the two glipizide metabolites found in human plasma — the 3-*cis*-hydroxycyclohexyl derivative and the 4-*trans*-hydroxycyclohexyl de-

rivative — interfered with the parent drug in the chromatogram; both eluted with the serum front.

Glibenclamide

Glibenclamide was extracted with benzene after acidification to pH 3. The mobile phase was 30% 0.01 *M* phosphate buffer (pH 3.5) in 70% methanol. Glibornuride served as internal standard.

Tolbutamide and chlorpropamide

The therapeutic serum concentrations of these two sulfonylureas are in the range of several $\mu\text{g/ml}$, while those of glipizide apparently are in the ng/ml range, i.e. 1000-fold less. This made it possible to use the glipizide extraction procedure also in the determination of tolbutamide and chlorpropamide in serum, even though the two compounds could not be used as internal standards. Indeed, this method was found to be very sensitive and rapid and to employ as little as 50 μl of serum. Toluene could be used as extraction medium instead of benzene; however, more serum was then needed, as the dried residue of toluene yielded some slowly eluting peaks that interfered significantly when detector sensitivity was high. Tolbutamide was used as internal standard for chlorpropamide and vice versa.

Interference of biguanides

Sulfonylureas are often used in combination with biguanides, such as metformin or phenformin. It was of particular importance, therefore, to assess whether biguanides would interfere with the determination of glipizide, glibenclamide, tolbutamide or chlorpropamide. No such interference was seen with either metformin or phenformin.

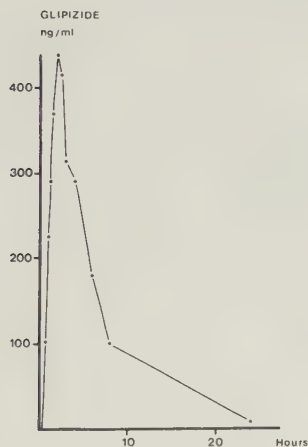


Fig. 3. Serum concentrations of glipizide during a 24-h period after ingestion of a single dose of 5 mg by a subject previously unexposed to the drug.

Glipizide in serum

Fig. 3. shows a glipizide concentration curve in serum from a subject who ingested 5 mg of glipizide. It is seen that the method allows detection of the drug over the whole 24-h period examined.

CONCLUSION

The presented method appears to be sufficiently selective, sensitive and rapid to allow accurate and precise measurements of the serum concentrations of glipizide and some other sulfonylureas during therapeutic conditions.

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Original Article

Bioavailability, Pharmacokinetics and Effects of Glipizide in Type 2 Diabetics

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Summary

The pharmacokinetics of glipizide were studied in 6 type 2 diabetics following single dose intravenous administration of 1mg, and oral administration of 2.5mg as a solution, a 2.5mg tablet and a 5mg tablet. The serum concentrations of the drug were measured by high pressure liquid chromatography. Glipizide showed a rapid distribution fitting a 2-compartment model. The distribution volume at assumed distribution equilibrium was small (10L), and the elimination half-life was short (2 to 4 hours). Gastrointestinal bioavailability was 100%. In one patient, glipizide absorption from tablets was retarded due to delayed tablet disintegration and drug dissolution.

Each dose of glipizide reduced blood glucose levels rapidly in all patients.

Glipizide is a potent, short acting sulphonylurea drug (Broden et al., 1979), which is being used increasingly in the treatment of type 2 diabetes. So far, there has been little information on its bioavailability and pharmacokinetics, due to the absence of appropriate methods for the determination of glipizide concentrations in body fluids. However, such a method was recently developed (Wåhlin-Boll and Melander, 1979), and the present study describes the pharmacokinetics of the drug in 6 type 2 diabetics following intravenous (1mg) and oral (2.5 and 5mg) administration. In addition, the changes in blood glucose levels following the different doses are given.

Material and Methods

Subjects

Three male and 3 female subjects with moderate type 2 diabetes mellitus were studied. Their mean age was 69 years (range 52-79), and the disease duration was 6 months to 13 years. Their mean bodyweight index (W/H^2) was $28 (\pm 7 \text{ SD}) \text{ kg/m}^2$ (range 22 to 41 kg/m^2). Their fasting blood glucose levels when entering the study ranged from 8.2 to 13.4 mmol/L with a mean of 10.1 mmol/L . Before the study, treatment had consisted of dietary regulation only, with-

out addition of any antidiabetic drugs or other medication. All patients had normal hepatic and renal function tests, and none had any endocrine disease apart from diabetes.

Experimental Procedure

The subjects were examined on 5 different occasions, in each case at 8am after fasting overnight. Blood samples were taken at regular intervals for analyses of blood glucose and serum glipizide concentrations before and after intake of breakfast (8.30am), lunch (11.30am) and dinner (4.30pm), both

without and with different dosage regimens of glipizide¹ (see below). The composition of the 3 meals was defined for each individual by a dietician, based on estimates of the individual caloric need. The protocol had been approved by the Ethical Committee of the University of Lund.

Glipizide Dosage

On day 1, testing was carried out without any glipizide administration; on day 2, 1mg glipizide was given intravenously; on day 4, a solution of 2.5mg glipizide in 200ml water was given orally; on day 7, a 2.5mg glipizide tablet was administered; and on day 9, a 5mg glipizide tablet was given. The drug was always administered 30 minutes before breakfast, and the tablets were taken with 200ml of water.

Blood Sampling

Serial blood samples were taken through an indwelling antebrachial venous polythene catheter. At each sampling, 1 to 2ml of blood was drawn and discarded before samples were collected. Samples for determination of blood glucose and serum glipizide concentrations were collected at -30 (before drug administration), 0 (at breakfast start), 30, 60, 90, 150, 210 (lunch), 240, 270, 300, 390, 510 (dinner), 540, 570, 630 and 690 minutes. During the examination after intravenous administration of 1mg glipizide, additional samples were obtained at 5, 10 and 20 minutes.

Serum Glipizide Concentrations

Glipizide concentrations in serum were determined by high pressure liquid chromatography (Wählin-Boll and Melander, 1979). The detection

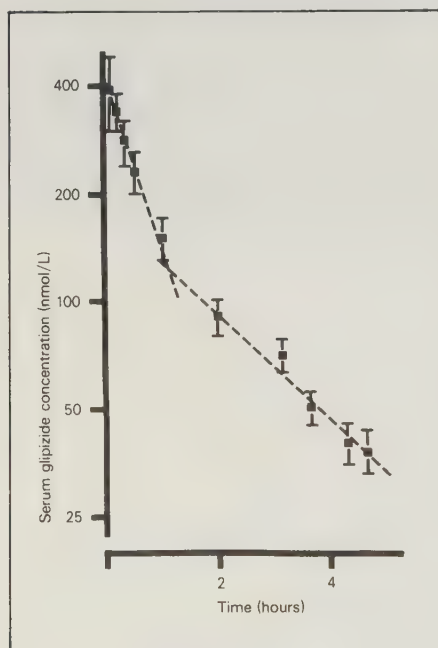


Fig. 1. Glipizide concentrations (mean $\pm$ SEM) in serum of 6 type 2 diabetics following a single intravenous dose of 1mg. The individual pharmacokinetic estimates are shown in table I.

¹ Min(i)diab[®] (Farmitalia-Carlo Erba).

Table 1. Pharmacokinetic parameters of glipizide in 6 type 2 diabetics following an intravenous dose of 1mg¹

Patient	α (h ⁻¹)	β (h ⁻¹)	C ₁ ⁰ (nmol/L)	V ₁ (L)	k ₁₂ (h ⁻¹)	k ₂₁ (h ⁻¹)	k _{el} (h ⁻¹)	Vd _{area} (L)	Clearance (L/h)	Duration of the α -phase (h)
1. HS	1.808	0.198	452	5.0	0.669	0.967	0.370	9.4	1.9	2.0
2. HH	6.932	0.258	987	2.3	4.515	1.320	1.355	13.2	3.4	1.0
3. AM	10.397	0.621	1156	1.9	4.943	4.702	1.373	4.6	2.9	0.5
4. AG	5.776	0.385	433	5.2	2.433	2.983	0.746	10.6	4.1	1.0
5. JA	1.664	0.249	294	7.6	0.420	1.124	0.369	11.3	2.8	1.0
6. GB	3.199	0.313	527	4.3	1.467	1.234	0.811	11.9	3.7	1.0
Mean	4.963	0.337	642	4.4	2.408	2.055	0.827	10.2	3.1	1.1
$\bar{x} \pm$ SD	+ 3.406	$\pm$ 0.153	+ 346	$\pm$ 2.1	$\pm$ 1.935	$\pm$ 1.492	$\pm$ 0.448	$\pm$ 3.0	$\pm$ 3.0	$\pm$ 0.5

1 Abbreviations:

- α = elimination rate constant during the α -phase.
- β = elimination rate constant during the β -phase.
- C₁⁰ = concentration of drug in compartment 1 at time 0.
- V₁ = volume of compartment 1.
- k₁₂ = first-order rate constant for transfer of drug from compartment 1 to compartment 2.
- k₂₁ = first-order rate constant for transfer of drug from compartment 2 to compartment 1.
- k_{el} = first-order rate constant for elimination of drug by all processes from compartment 1.

$$Vd_{area} = \frac{Dose}{\beta \text{ AUC}_{0-\infty}}$$

$$Clearance = \frac{Dose}{\text{AUC}_{0-\infty}}$$

AUC = area under the concentration-time curve.

Table II. Pharmacokinetic parameters of glipizide in 6 type 2 diabetics following single doses of (a) 1mg intravenously; (b) 2.5mg solution in 200ml water; (c) 2.5mg tablet with 200ml water; and (d) 5mg tablet with 200ml water¹

Patient	C _{max} (nmol/L)				t _{max} (h)				t _{1/2} (h)				AUC (nmol × h × L ⁻¹)			
	b	c	d		b	c	d		a	b	c	d	a	b	c	d
1. HS	745	424	851		0.5	1.0	1.5		3.5	2.1	3.6	3.9	1202	2303	2646	5585
2. HH	364	171	242		1.0	2.5	1.5		2.7	2.5	3.1	3.0	661	1508	1425	2424
3. AM	1044	790	685		0.5	1.0	1.0		1.1	2.5	2.2	2.4	786	2041	1975	1706
4. AG	534	440	1035		0.5	1.0	1.0		1.8	2m3	2.7	2.6	552	1600	1472	3717
5. JA	514	494	1109		0.5	1.0	1.5		2.8	2.8	3.1	3.0	795	2157	2552	5699
6. GB	822	292	462		0.5	25	3.4		2.2	2.6	3.3	3.5	601	2158	1912	3872

Abbreviations:

C_{max} = peak concentration.

t_{max} = time to peak concentration.

t_{1/2} = elimination half-life.

AUC = area under the serum concentration-time curve.

limit was 10ng/ml, using an injection volume of 20 μ l.

Blood Glucose Concentrations

Blood glucose concentrations were determined by the glucose oxidase method (Marks, 1959).

Calculations

The blood glucose and serum glipizide concentrations were plotted against time. Areas under the concentration-time curves (AUC) were calculated according to the trapezoidal rule. For the pharmacokinetic

analysis of the intravenous 1mg dose, calculations according to Wagner were employed (Wagner, 1975). The statistical significance of differences was calculated by the Wilcoxon signed rank test.

Results

Serum Glipizide Concentrations

Following the administration of 1mg of glipizide intravenously (fig. 1), the serum concentration curves suggested that the drug was distributed according to a 2-compartment model. The early α -phase was always completed within 2 hours (mean 1.1 hours) and had a mean half-life of 13 minutes. The subsequent β -phase

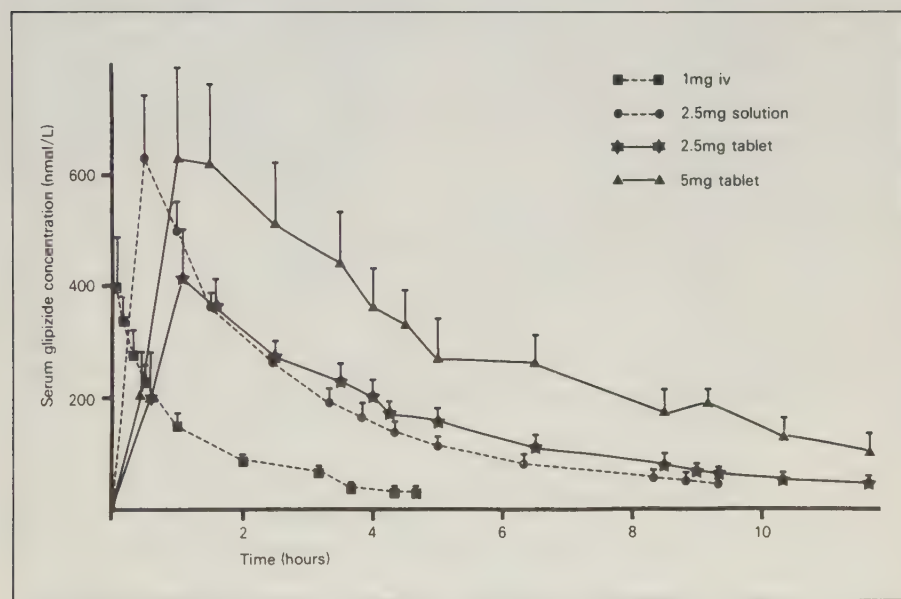


Fig. 2. Glipizide concentrations (mean $\pm$ SEM) in serum of 6 type 2 diabetics following a single intravenous dose of 1mg, and oral doses of a 2.5mg solution in 200ml water, a 2.5mg tablet with 200ml water and a 5mg tablet with 200ml water. The individual pharmacokinetic estimates are shown in table II.

had a mean half-life of 2.4 hours (range 1.1-3.5). The distribution volume of the central compartment ranged from 1.9 to 7.6L with a mean of 4.4L. The distribution volume at assumed distribution equilibrium ($V_{d\beta}$) had a mean value of 10.2L (range 4.6-13.2L). The mean overall clearance of the drug was 3.1L/hour (range 1.9-4.1). Table I shows the individual pharmacokinetic data. Table II gives the individual estimates of the AUC and the terminal half-lives.

Following intake of a 200ml solution of 2.5mg of glipizide, the peak concentrations (C_{max}) were significantly higher ($p < 0.05$) than after intake of a 2.5mg

tablet (fig. 2). On the other hand, neither the terminal half-life nor the AUC differed significantly. Table II shows the individual data of $t_{1/2}$, t_{max} , C_{max} and AUC ($0 \rightarrow \infty$).

After intake of a 5mg tablet (fig. 2), the mean AUC value was about twice as great as that following the 2.5mg doses (table II).

After intake of glipizide tablets, the interindividual variation of the drug concentrations increased with dose. After intake of tablets, but not of solution, 1 patient (2, HH) showed a highly aberrant concentration profile, with a long lasting plateau-like level (fig. 3).

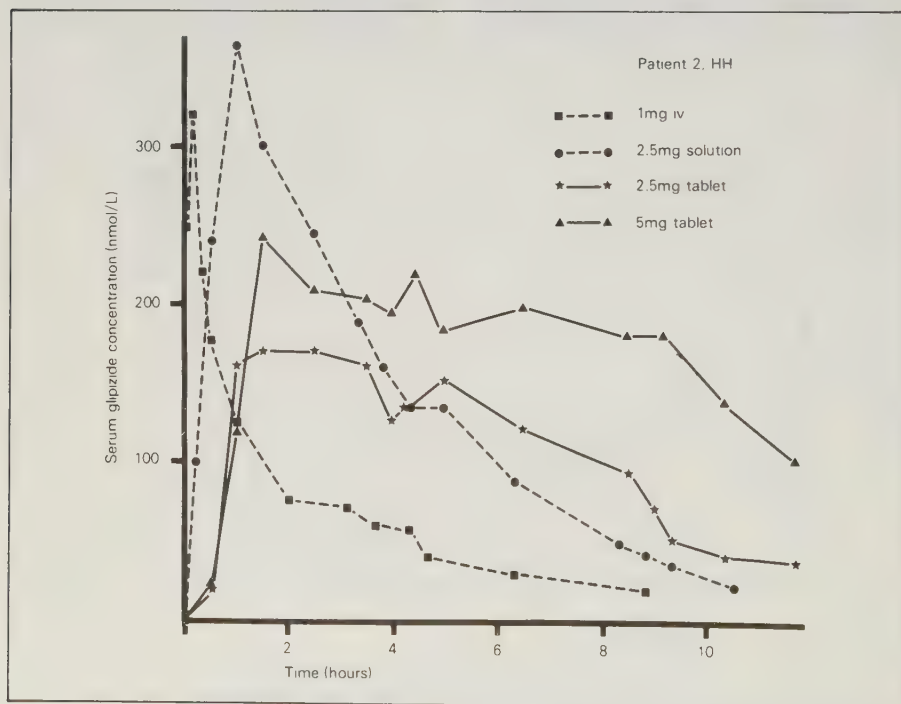


Fig. 3. Serum glipizide concentrations in 1 diabetic patient (2, HH) following single doses of the drug as described in figure 2. Note the major differences between solution and tablets.

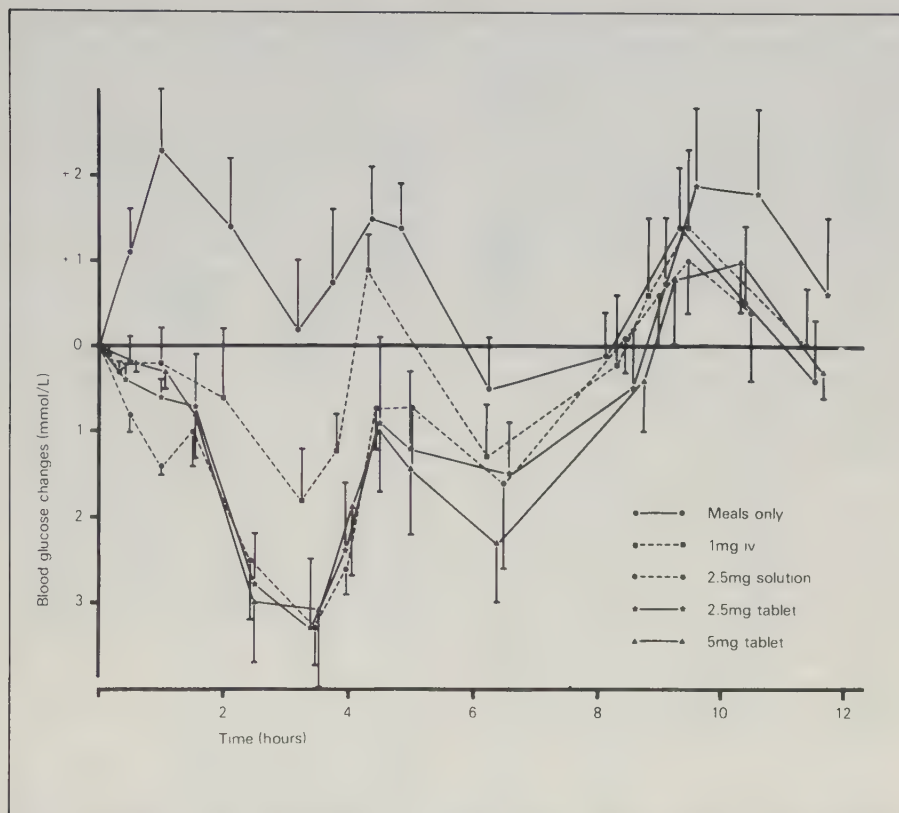


Fig. 4. Blood glucose (mean $\pm$ SEM) changes in 6 type 2 diabetics following a standardised breakfast, lunch and dinner with and without single glipizide doses as described in figure 2.

After correction for the dose differences, the mean ratios of AUC_{oral}/AUC_{iv} were 1.066, 1.062 and 1.028, respectively.

In 1 patient (3, AM) the (uncorrected) AUC_{5mg} was the same as that of $AUC_{2.5mg}$, and the concentration profiles were overlapping. However, it is possible that this patient had been given 2.5mg instead of 5mg by mistake.

Blood Glucose Concentrations

Figure 4 shows the mean blood glucose changes following breakfast, lunch and dinner with and without glipizide administration. The lower panel of figure 5 demonstrates the total AUC values of blood glucose, i.e. the area above 0mmol/L, from 0 to 11 hours. The upper panel shows the net areas, i.e. the

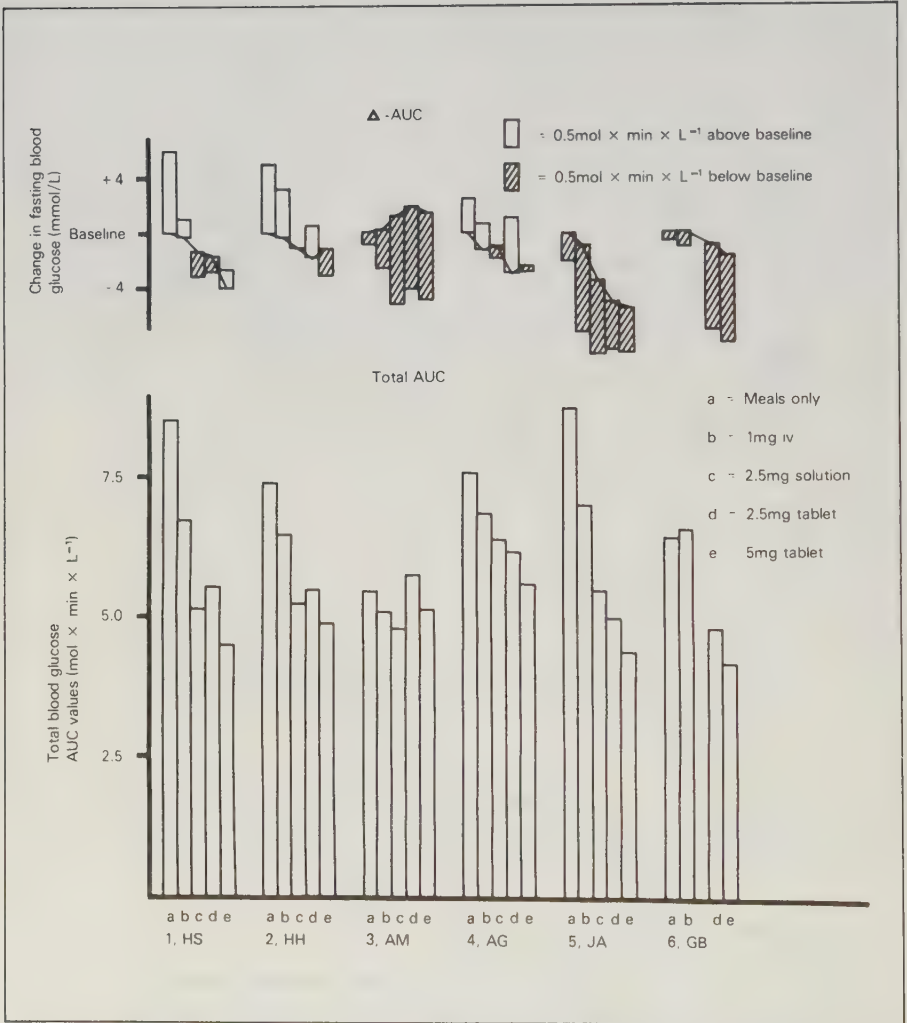


Fig. 5. Upper panel: Net areas (above fasting level minus below fasting level) of blood glucose, and change in fasting blood glucose levels (indicated by a line from 'baseline') in 6 type 2 diabetics given single glipizide doses as described in figure 2. Lower panel: Total blood glucose areas (above 0mmol/L) in the same patients. [In 1 patient (6. GB), blood glucose values from the test with the 2.5mg solution (c) were accidentally lost].

area above minus the area below the fasting blood glucose levels, and the line between the bars indicates the change in fasting blood glucose levels between the different tests.

The total AUC values decreased ($p < 0.05$) with increasing glipizide dose. 1 patient (3, AM) was an exception to this, and she also differed from the other patients in that her fasting blood glucose level tended to increase with time, whereas the opposite was seen in the other 5 patients. Overall, the mean fasting blood glucose levels were 10.1 (no glipizide), 9.7 (1 mg intravenously), 9.2 (2.5 mg oral solution), 8.5 (2.5 mg tablet) and 7.9 (5 mg tablet) mmol/L (reduction not significant).

Even though the total AUC in patient No. 3 did not decrease, there was a reduction in the net area, as was also the general tendency seen in the other 5 patients. Indeed, each glipizide dose significantly ($p < 0.05$) reduced the net areas, the means being +431 (no glipizide), -139 (1 mg intravenously), -715 (2.5 mg oral solution), -427 (2.5 mg tablet) and -702 (5 mg tablet) mmol $\times$ min $\times$ L⁻¹. The difference in effect between the different glipizide doses, on the other hand, did not reach statistical significance.

Discussion

To date there has been little information on glipizide pharmacokinetics in man based upon measurements of unlabelled glipizide. Moreover, the pharmacokinetics of glipizide have mainly been studied in healthy volunteers, not in the target population, i.e. type 2 diabetics. The present study employed a recently described high-pressure liquid chromatography technique that is highly selective for glipizide (Wählin-Boll and Melander, 1979), and the subjects were all elderly type 2 diabetics.

The findings confirm previous observations with ¹⁴C-labelled glipizide (Balant et al., 1975) on the rates of absorption and elimination of the drug following single dose administration: the peak concentration was usually reached within 1 hour, and the elimina-

tion half-life was calculated at 1.1 to 3.9 hours, the highest value mostly being seen after intake of the largest dose.

As the AUC_{oral}/AUC_{iv} ratio was close to, and often exceeded, 100% with each of the oral doses, it is reasonable to assume that the gastrointestinal absorption of glipizide is complete, that there is essentially no first-pass biotransformation of the drug, and, consequently, that the oral bioavailability of glipizide is about 100%, not only from the solution but also from the 2.5 and 5 mg tablets.

In 1 patient, the absorption of glipizide from the tablets, but not from the solution, seemed to be considerably retarded, and the overall variation in absorption from the tablets appeared to be more pronounced than that from the solution. This suggests that tablet disintegration and glipizide dissolution may be a significant factor for the rate (but not the extent) of glipizide absorption.

The pharmacokinetics of glipizide following a single intravenous dose (1 mg) of the drug showed a rapid distribution fitting a 2-compartment model. The distribution volume of the central compartment was only about 4.5 L, and the distribution volume at assumed distribution equilibrium was also small (about 10 L). The small distribution volume may be a reflection of the high degree of plasma protein binding of glipizide; a figure of 98% binding has been obtained by equilibrium dialysis (Crookes and Brown, 1975).

The high clearance value, the small distribution volume and the short elimination half-life strongly support the view that glipizide is very rapidly eliminated from the body. This is in agreement with previous observations (Balant et al., 1975), and it appears that the rapid elimination results from extensive metabolism (Brogden et al., 1979). There was a tendency toward longer elimination half-lives with larger doses, but there was no distinct sign of a slowly equilibrating deep compartment, as suggested by studies employing radiolabelled glipizide (Balant et al., 1975). However, the possible existence of such a compartment cannot be ruled out by a single-dose study, but should be looked for following multiple

dosing with glipizide. This could also tell whether the deep compartment would have clinical relevance.

As regards the influence of glipizide on blood glucose levels, it should be emphasised that the patients studied were fairly well regulated on long term dietary restrictions and had no overt need of sulphonylurea treatment. This is the reason why the dosage schedule was not randomised; it was judged as being potentially dangerous, and hence unethical, to give the high dose until after the effect of a low dose had been tested.

Even though the different oral glipizide doses were given 2 days apart, there was a gradual, albeit statistically insignificant, reduction of the fasting blood glucose levels with time. This complicates the interpretation of the response to glipizide, because the effect of a sulphonylurea depends on the blood glucose concentration (Pfeifer et al., 1980). As glipizide is very short acting (Brogden et al., 1979) and as the serum drug concentrations had returned to zero well before the next dose was administered, it is unlikely that there was a carryover effect from one dose to the next.

It is more likely that the improved fasting blood glucose levels resulted from enhanced compliance with the dietary regulations, as a consequence of the fact that the patients came into repeated and long term contact with the day-care centre. The one patient who showed an increased level of fasting blood glucose during drug exposure contracted a common cold on day 4.

The total blood glucose AUC, i.e. over 0 mmol/L , showed an increased reduction with increasing dose. As the fasting blood glucose level was gradually diminished, the AUC reduction following one dose could have resulted from the preceding dose and/or preceding dietary improvement. This cannot be the sole explanation, however, as the net areas, i.e. the area above minus the area below the fasting blood glucose level, were also significantly reduced. Indeed, the net areas were diminished even in the single patient whose fasting blood glucose level increased rather than decreased. Thus, it can be stated that each dose of glipizide was effective in all patients.

Therapeutic Implications

Glipizide is a potent and rapid-acting sulphonylurea drug with 100% oral systemic availability. It has a small volume of distribution and seems to be rapidly eliminated. Accordingly, glipizide should be useful in the treatment of type 2 diabetes, and the risk of long lasting hypoglycaemia should be minor.

Acknowledgements

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STEADY-STATE ELIMINATION RATE AND ENTEROHEPATIC RECIRCULATION OF GLIPIZIDE

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ABSTRACT

Glipizide is a potent- and rapid-acting sulfonylurea with complete bioavailability, a small volume of distribution, low clearance, and a single-dose half-life of less than 5 h. The current study examined the terminal elimination half-life during and after long-term treatment to see whether the elimination of glipizide is prolonged during steady-state conditions. In addition, the study investigated the possibility of an enterohepatic recirculation of this drug.

The terminal elimination half-life was found to remain below 5 h at steady state, excluding saturation and cumulation processes in the long-term handling of glipizide, and supporting the view that glipizide is unlikely to promote long-lasting hypoglycemia. Secondary plasma concentration peaks were found following meals, and there were secondary peaks or transient slowdowns in glipizide elimination as well as minor blood glucose reductions following injection of cholecystokinin. This suggests that enterohepatic recirculation can occur following meals, which may help to explain why glipizide is particularly efficient during postprandial conditions.

Glipizide is a potent and rapid-acting sulfonylurea with complete bioavailability (Wählin-Boll et al. 1982; Melander & Wählin-Boll, 1983). Two long-term studies have shown that once-daily administration can be therapeutically equal to thrice-daily intake (Östman et al. 1981, Wählin-Boll et al., submitted). This may seem odd as the plasma elimination half-life of glipizide is below 5 h following single doses of 1-5 mg (Wählin-Boll et al. 1982), and it may be suspected, therefore, that the elimination of glipizide would be slower during steady-state conditions. Another possibility is that glipizide undergoes enterohepatic recirculation, an idea supported by the fact that the apparent oral bioavailability of glipizide sometimes exceeds 100 per cent (Wählin-Boll et al. 1982). Moreover, secondary peaks in the time-

concentration curves have been observed following meals (Wahlin-Boll et al. 1982; Pentikäinen et al. 1983), suggesting recirculation of the drug. Meal-induced recirculation of the drug might also help to explain why glipizide is particularly efficient in reducing blood glucose increases following meals (Wahlin-Boll et al. in press).

In the current study, the terminal elimination half-life of glipizide in serum was measured in patients with type 2 diabetes both during and after cessation of long-term treatment with the drug. In addition, the influence of exogenous cholecystokinin on the single-dose kinetics of glipizide was studied in healthy volunteers, to assess the possibility of an enterohepatic recirculation of the drug.

SUBJECTS AND METHODS

Patients

Twelve (6 female, 6 male) patients with type 2 diabetes were studied with respect to the long-term elimination half-life of glipizide. Five of them were regular attenders at the Medicine Clinic of Lund University Hospital, while 7 were regular attenders at the Medicine Clinic of Mölndal Hospital, south of Göteborg. All subjects gave informed consent to participate. Their mean age ($\pm$ SD) was 58 ± 8.8 years, range 46-71, their body weights 68.7 ± 11.9 kg, range 52.5-94.5, and their disease duration was 2-16 years. None had any endocrine disease apart from diabetes. Two patients were treated with selective beta blockers, and this medication was kept unaltered during the study. No patient took any (other) medication known to influence blood glucose or glipizide kinetics.

Before the study, all patients had been treated with glipizide* for a minimum of one month. The 5 patients recruited in Lund had developed secondary failure, justifying withdrawal of glipizide and initiation of insulin

treatment. Blood samples were repeatedly taken for an average of 16.5 hours after the last dose as specified in Results.

The 7 patients from Mölndal were being treated with 5 (n=5) or 10 mg (n=2) glipizide* once daily. From these patients, blood samples for glipizide determinations were taken every other hour from 7 a.m. to 8 p.m. and, in addition, at midnight, 4 a.m. and 7 a.m.

A 56-year-old female patient at the Department of Medicine, Uppsala University Hospital, who had both type 2 diabetes and hepatic cirrhosis, was studied after ingestion of a single 10 mg dose of glipizide. Blood samples were obtained for 0-12 h.

The protocols had been approved by the local committees on ethics.

Volunteers

Six healthy male volunteers, with a mean ($\pm$ SD) age of 33.7 ± 8.2 years, range 21-43, and a mean weight of 77.0 ± 5.2 kg, range 70.5-84.5, participated in the study. All gave written consent after extensive information, and the protocol was approved by the local committee on ethics. The volunteers came to the laboratory in the morning, after having fasted overnight (10 p.m. to 8 a.m.). After an initial blood sample they ingested a 5 mg tablet of glipizide* together with 100 ml of water. During continued fasting, blood samples were taken every half-hour for three hours after tablet intake. An i.v. injection of porcine cholecystokinin (CCK)**^(R), 1 unit per kg bodyweight, was then given. Additional blood samples were taken at 15, 25 and 35 minutes after the CCK injection, and then every half-hour until 5 hours after tablet intake. During this time no food, liquid or smoking was allowed.

* Min(i)diab^(R), Farmitalia Carlo Erba, Milano, Italy

**Cholecystokinin, Kabi Vitrum, Stockholm, Sweden

Chemical analyses

Serum glipizide concentrations were assayed by high-pressure liquid chromatography (Wählin-Boll & Melander 1979). Blood glucose concentrations were determined by a glucose electrode (Yellow Spring Instrument Glucose Analyzer).

Calculations

The drug elimination half-life was calculated by regression analysis.

RESULTS

Terminal elimination half-life of glipizide

The mean terminal elimination half-life of glipizide in diabetic subjects following withdrawal of long-term treatment was 3.54 ± 0.69 h, range 2.38 - 4.63 h. This calculation was made with great accuracy; the mean correlation coefficient was 0.991 ± 0.009 (range 0.975 - 0.999). The elimination of glipizide was followed during 3-6 half-lives (mean of 5.1 ± 0.9 half-lives, range 3.4 - 6.4). The individual elimination half-lives are given in Table I.

Secondary peaks following meals

Fig. 1 shows single-dose curves from patients investigated in a previous study (Wählin-Boll et al. 1982). It can be seen that the elimination pattern of plasma glipizide is altered shortly after lunch intake, with the appearance of secondary peaks.

Fig. 2 shows the serum glipizide concentration curve following a single 10 mg dose in a patient with hepatic cirrhosis in addition to type 2 diabetes. She had a large secondary peak at lunch time, exceeding the primary peak.

In addition, there was a third increase at dinner time.

Influence of exogenous cholecystokinin on single-dose kinetics of glipizide

Following i.v. injection of CCK, 3 volunteers had a small but distinct second peak in their serum glipizide elimination curves, and 2 other volunteers showed a transient slowdown in glipizide elimination. The mean serum glipizide curves can be seen in Fig. 3.

The CCK-evoked changes in the plasma glipizide were accompanied by minor changes in the blood glucose levels. In 3 volunteers the blood glucose was lowered by 0.3 mmol/l 25-35 min after the CCK injection, and in 2 volunteers by 0.1 mmol/l 25 min after the CCK injection. The mean blood glucose curves can be seen in Fig 2.

DISCUSSION

Glipizide is a lipophilic, weakly acidic drug with a high degree of binding to plasma albumin (Crooks & Brown 1975). Accordingly, it has a very small apparent distribution volume, about 10 l, reflecting that most of the drug is present in plasma (Wählin-Boll et al. 1982). Hence, glipizide elimination from the body should almost equal that from plasma. This is supported by previous data; a 1 mg dose disappeared from plasma within 4 h and a 2.5-5 mg dose within 8-12 h, and the corresponding effect durations were similar (Wählin-Boll et al. 1982). This relation emphasizes the importance of an accurate estimate of the plasma elimination half-life of glipizide.

A single-dose study employing a selective and sensitive HPLC technique has indicated that the elimination half-life is less than 5 h (Wählin-Boll et al. 1982), but an earlier single-dose study measuring the plasma decay of total radioactivity following administration of ¹⁴C-labelled glipizide reported a

terminal half-life exceeding 6 h, suggesting the existence of a deep compartment from which glipizide is slowly eliminated (Balant et al. 1975). However, the current long-term study gave no evidence of such a process; on the contrary, the terminal elimination half-life of glipizide remained below 5 h, and the accuracy of this measurement was high. Indeed, the HPLC method allowed determinations of glipizide in plasma for a period corresponding to about 5 half-lives, with excellent correlation coefficients. It seems likely, therefore, that the terminal elimination half-life of glipizide is not longer than 5 h, and that there is no cumulation or saturation process involved in the handling of glipizide. The longer half-life previously reported (Balant et al. 1975) most probably reflects elimination of glipizide metabolites, a possibility also forwarded by these authors (Balant et al. 1975).

Although the half-life of glipizide is 5 h or less, two different studies have shown that, in daily doses of 7.5 mg or more, a once-daily regimen is therapeutically equivalent with a thrice-daily regimen (Östman et al. 1981; Wählin-Boll et al., submitted). This may relate to the fact that 7.5 mg or more once-daily is sufficient to keep the plasma level of glipizide over the threshold concentration for at least 12 h, and it is also likely that glipizide is mainly active during postprandial conditions (Wählin-Boll et al., in press). For this reason, it is of particular interest that the present and previous studies have observed secondary peaks in the plasma concentration profiles of glipizide, co-inciding with intake of meals. Assuming that unmetabolised glipizide undergoes enterohepatic recirculation, the secondary peaks could reflect reabsorption of glipizide following release from the gall bladder, subsequent to meal intake. Although the changes in glipizide and glucose concentrations following cholecystokinin injection were slight, their pattern is consistent with enterohepatic recirculation and reabsorption of glipizide.

A most impressive recirculation picture, with the secondary peak exceeding the primary peak and with a third increase at dinner time, was seen in a

patient with cirrhosis. This could be explained by a reduced hepatic capacity to metabolise glipizide, with more drug being excreted in the bile.

To summarize, glipizide has rapid absorption, complete bioavailability, a small volume of distribution and a rapid elimination with a terminal half-life of 5 h or less. This makes it unreliable to promote long-lasting hypoglycemia. Glipizide may undergo enterohepatic recirculation to a minor degree, with reabsorption following meals. This may help to explain why glipizide is particularly efficient during postprandial conditions.

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TABLE I

Terminal plasma elimination half-lives of glipizide in 12 patients with type 2 diabetes following cessation of long-term treatment with the drug.

Patient no.	Sex	$t_{1/2}$ h	corr. coeff. of $t_{1/2}$	no. of half- lives followed
1	M	3.09	0.996	6.2
2	M	4.28	0.996	4.6
3	M	3.75	0.996	4.8
4	M	4.63	0.990	3.9
5	M	3.13	0.976	5.8
6	M	2.38	0.991	3.4
7	F	2.67	0.999	5.6
8	F	3.26	0.990	5.8
9	F	3.42	0.995	6.4
10	F	3.52	0.975	5.4
11	F	4.02	0.999	4.5
12	F	4.30	0.984	4.4
Mean $\pm$ SD		3.54 $\pm$ 0.69	0.991 $\pm$ 0.009	5.1 $\pm$ 0.9

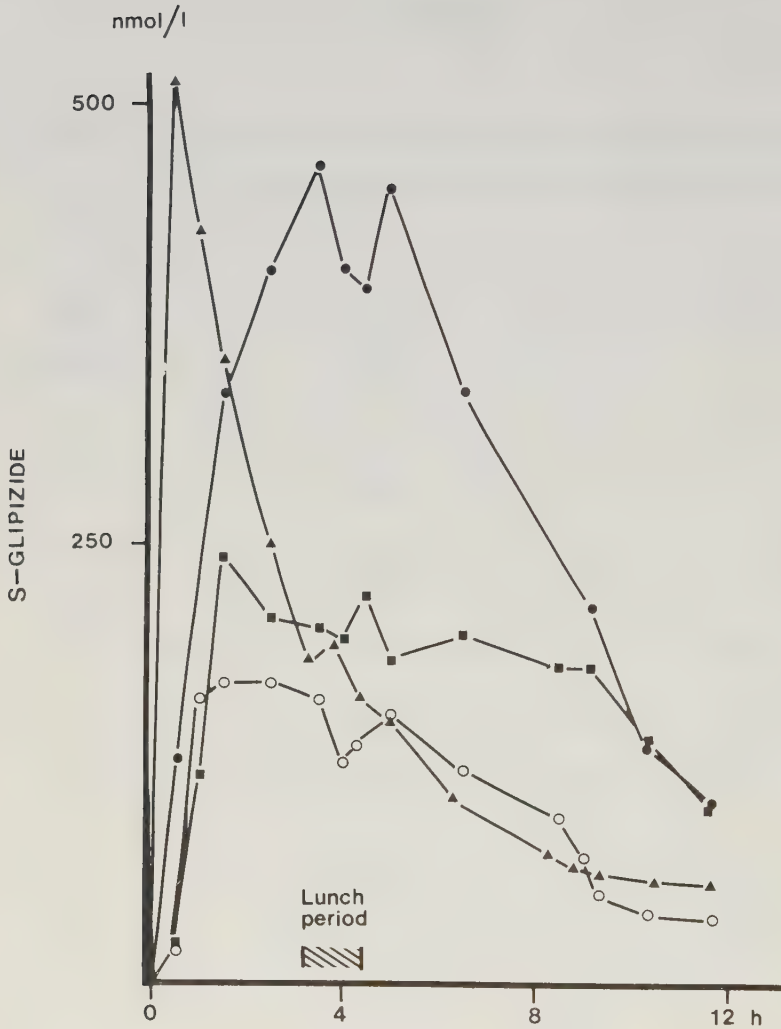


Fig. 1

Four individual glipizide serum concentration curves following oral intake of single doses (2.5 mg in two, 5 mg in two cases) at time 0. Note the secondary peaks appearing at lunch time.



Fig. 2

Serum glipizide concentration curve in a patient with hepatic cirrhosis. Note the pronounced secondary peak at lunch time and the third increase at dinner time.

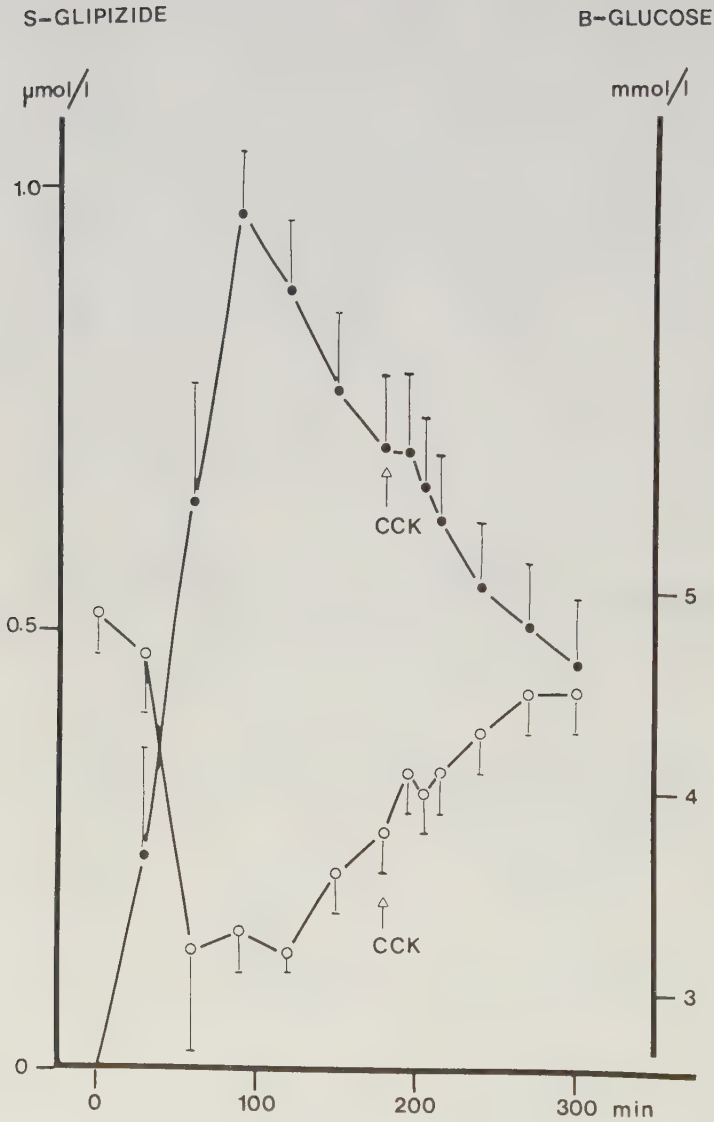


Fig. 3

Serum glipizide and blood glucose levels in 6 individuals following oral intake of 5 mg glipizide at time 0 and receiving one i.v. injection of cholecystokinin (CCK) as indicated by arrow. Mean $\pm$ SEM indicated.

Influence of Food Intake on the Absorption and Effect of Glipizide in Diabetics and in Healthy Subjects

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Summary. The influence of a standardized breakfast on the single dose (5 mg) kinetics and effects of glipizide was examined in 9 healthy volunteers and in 14 diabetics not previously exposed to a sulfonylurea. In the volunteers, glipizide caused an increase in plasma insulin and a reduction in blood glucose both during continued fasting and when the drug was taken with the breakfast. Food intake did not influence the peak concentration, the elimination half-life or the bioavailability of the drug. However, food intake significantly delayed the absorption of glipizide by about 0.5 h. In the patients, glipizide produced a significant increase in plasma insulin and a significant diminution of the rise in blood glucose in response to the meal. Starting at breakfast and for 45 min thereafter serum glipizide concentrations were significantly higher when the drug was taken 0.5 h before the meal, than when ingested concurrently with it. With the former treatment, the increase in plasma insulin occurred earlier and the blood glucose reduction was pronouncedly greater than with the latter treatment. As the absorption of glipizide may be delayed by concurrent breakfast, this may help to explain, why the administration of glipizide 0.5 h before breakfast led to a more appropriate relation between the serum concentration of the drug and the metabolic impact of the meal, thereby promoting more appropriate insulin release and better glucose disposition than after concurrent intake of the drug and breakfast.

Key words: glipizide, diabetes; food intake, blood glucose, blood insulin, pharmacodynamics, pharmacokinetics

renone [4], phenytoin [5], dicoumarol [6], carbamazepine [7], hydrochlorothiazide [8], nitrofurantoin [9] and erythromycin stearate [10] is enhanced by food, whereas that of isoniazid [11], rifampicin [12], ampicillin [13] and atenolol [14] is reduced. As appropriate diet regulation is a major function in the control of diabetes, the relation between food intake and administration of oral antidiabetic drugs should be of particular importance. As far as tolbutamide, chlorpropamide and glibenclamide are concerned, no significant influence on drug bioavailability has been recorded [15, 16]. Nevertheless, their effects may depend upon the relative timing of intake of the drug and food [15, 16]. In addition, a recent study [17] has shown that the blood glucose-reducing effect of glipizide, a novel, rapid-acting and potent sulfonylurea, is more pronounced if the drug is taken 0.5 h before than when taken together with breakfast. The present study deals with the influence of food intake on the kinetics and effect of glipizide both in healthy subjects and in diabetics.

Material and Methods

Healthy Volunteers

Nine healthy male volunteers, with a mean ($\pm$ SD) age of 26 ($\pm$ 1.9) years (range 23–28), and mean ideal body weight of 94.1 ($\pm$ 5.9) per cent (range 85.6–102.8) participated in the study. Each subject was extensively informed and gave written consent to it. After an overnight fast (10 p.m. to 8 a.m.), each subject came to the laboratory on three different mornings, at intervals of at least one week. They were then given a) a standardized breakfast of 1800 kJ [cf. 15, 16]; b) glipizide 5 mg together with the meal; and c) glipizide 5 mg alone. The drug was given as single 5 mg tablets, all of the same brand and

Food intake has been found to influence the disposition of several drugs [1]. Thus, the bioavailability of hydralazine [2], propranolol, metoprolol [3], can-

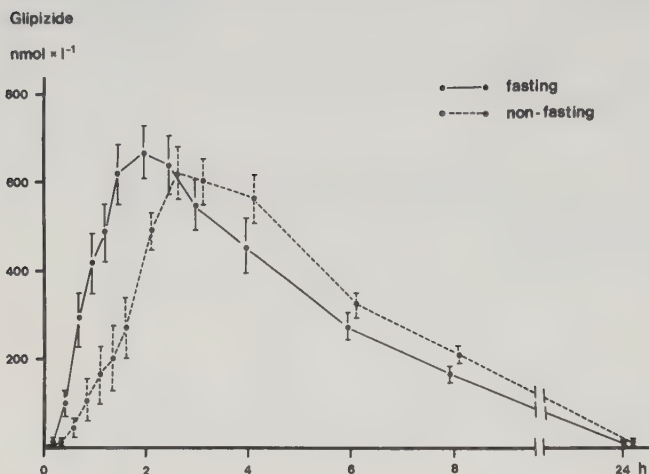


Fig. 1. Serum concentration (mean $\pm$ SEM) of glipizide in 9 healthy volunteers following ingestion of 5 mg in the fasting and in the non-fasting (standardized breakfast) states. The delay of about 40 min in the time to reach peak concentration was significant ($p < 0.05$)

batch (Min(i)diab®, Carlo Erba, Milano, Italy). Apart from the meal, no food or liquid was allowed until 4 h after initiation of the experiment. Thus, when glipizide alone was given, the subjects fasted for 4 h in addition to the preceding 10 h (overnight) fasting period.

Diabetic Patients

The study initially included 11 male and 4 female patients with maturity-onset diabetes mellitus, treated by conventional dietary regulation and without any antidiabetic agent¹. One male was excluded because glipizide in his serum could not be measured due to interfering substances. Characteristics of the patients and the study design have been described previously [17].

Laboratory Procedures

Serial blood samples were obtained at intervals specified in Results. The serum concentration of glipizide was measured by high-pressure liquid chromatography [18]. Blood glucose concentration in patients was measured by the glucose oxidase

Table 1. Kinetic parameters (mean $\pm$ SEM) of glipizide in nine healthy volunteers following a single oral dose (5 mg) on an empty stomach and together with a standardized breakfast. C_{max} = peak concentration, t_{max} = time to peak concentration, $t_{1/2}$ = elimination half-life, AUC = area under the serum concentration curve.

	C_{max} [nmol/l]	t_{max} [min]	$t_{1/2}$ [h]	AUC (0–8 h) [nmol $\times$ h $\times$ l ⁻¹]
Fasting:	720 $\pm$ 68	120 $\pm$ 9	4.1 $\pm$ 0.6	3004 $\pm$ 281
Non-Fasting:	715 $\pm$ 51	160 $\pm$ 14	4.0 $\pm$ 0.5	2912 $\pm$ 106
Significance of difference (paired <i>t</i> -test) between fasting and non-fasting conditions:	N.S.	$p < 0.05$	N.S.	N.S.

method [19] with a minor modification [20]. In volunteers the hexokinase method [21] was employed. Plasma insulin was analysed by a commercial solid phase radioimmunoassay (Phadebas Insulin-Test®, Pharmacia, Uppsala, Sweden).

Calculations

Peak concentration (C_{max}) was defined as the maximum recorded value. The drug elimination half-lives in volunteers were calculated by regression analysis, and the areas under the glipizide concentration curve (AUC) values (from 0 to 8 h) were estimated by the trapezoidal rule. There was a tendency toward a shift

¹ The glucose and insulin responses of these 15 patients to glipizide 5 mg given with and before breakfast have been published previously (Acta Med. Scand. 203: 211, 1978). The data are republished here with the permission of the Editor-in-Chief of Acta Medica Scandinavica

in the half-life after 6 h, and the 24 h concentrations were sometimes too low to permit accurate measurement of glipizide. Hence, residual AUC ($8 \rightarrow \infty$) could not safely be calculated.

Statistics

Statistical analysis was done with Student's paired *t*-test.

Results

Healthy Volunteers

Serum concentrations of glipizide in the volunteers following ingestion of 5 mg during fasting and non-fasting conditions are shown in Fig. 1, and the kinetic estimates are given in Table 1. It appears that food intake did not influence the peak concentrations, elimination half-lives or AUC values. However, in the non-fasting state the time to reach peak concentration was prolonged by approximately 40 min ($p < 0.05$).

When glipizide was added to the breakfast, higher plasma insulin concentrations than after the meal alone were reached at 60, 120 ($p < 0.05$) and 150 min ($p < 0.01$) (Fig. 2, lower panel). Glipizide given during continued fasting caused an increase in plasma insulin to above the initial level at 60 ($p < 0.05$), 75 ($p < 0.01$) and 90 min ($p < 0.05$).

When glipizide was added to the breakfast, the rise in blood glucose in response to the meal was reduced at 45 ($p < 0.01$), 60 ($p < 0.05$), 75, 90 ($p < 0.01$), 120, 150 ($p < 0.001$) and 180 min ($p < 0.01$) (Fig. 2, upper panel). When the drug was given during continued fasting, blood glucose concentrations were reduced ($p < 0.001$) from 45 to 180 min as compared to the initial level (4.1 ± 0.1 mmol/l). A mean nadir of $2.2 (\pm 0.2)$ mmol/l was reached at 75 min (Fig. 2, upper panel).

Diabetic Patients

The serum concentrations of glipizide in the patients are shown in Fig. 3. When the drug was given 0.5 h before breakfast, glipizide concentrations were greater not only at the start of breakfast ($p < 0.01$) but also for the subsequent 45 min ($p < 0.01$ – $p < 0.001$), as compared to concurrent intake of drug and breakfast.

As compared to breakfast only, concurrent intake of glipizide gave higher ($p < 0.05$ – 0.01) insulin concentrations at 15 to 120 min (Fig. 4, lower panel). When the drug was given half an hour before the

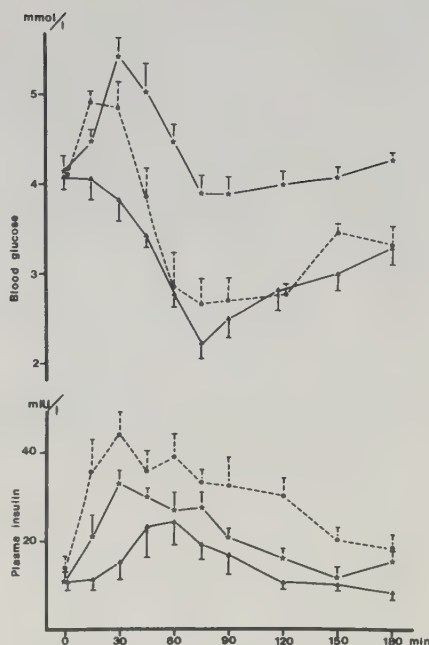


Fig. 2. Plasma insulin (lower panel) and blood glucose (upper panel) concentrations (mean and SEM) in 9 healthy volunteers following administration of standardized breakfast (*—*), glipizide 5 mg during continued fasting (▲—▲) and glipizide 5 mg taken together with the breakfast (●—●) (cf. Fig. 1)

meal, the plasma insulin increase occurred earlier; the insulin concentrations were higher when the meal was begun ($p < 0.05$), and 5 ($p < 0.01$) and 10 min ($p < 0.001$) after the meal (Fig. 4, lower panel).

The mean fasting blood glucose level was not significantly different in the three studies. When glipizide was given together with the meal, the blood glucose increase was lower at 60 to and 90–180 min ($p < 0.01$) than when only the meal was given (Fig. 4, upper panel). When glipizide was given 0.5 h before the meal, the increase in blood glucose in response to the meal was already reduced by 45 min ($p < 0.01$) and at all observation times up to 180 min ($p < 0.001$) (Fig. 4, upper panel). The difference recorded between preprandial and prandial administration of the drug was significant at 90 ($p < 0.01$) and 120 min ($p < 0.05$). In addition, the decremental

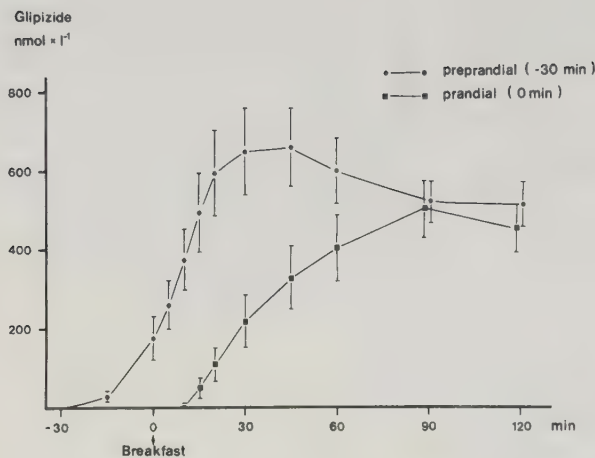


Fig. 3. Serum concentrations (mean and SEM) of glipizide in 14 diabetic patients, not previously exposed to a sulfonylurea. The drug (5 mg) was ingested 0.5 h before and concurrently with a standardized breakfast

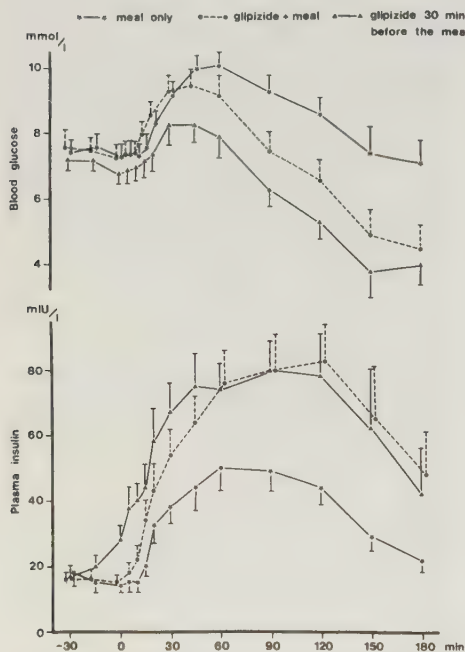


Fig. 4. Plasma insulin (lower panel) and blood glucose (upper panel) concentrations (mean and SEM) in 14 diabetic patients given a standardized breakfast, glipizide 5 mg together with the breakfast, and glipizide 5 mg 0.5 h before the breakfast. (Data re-published from Acta Med. Scand. by permission of the Editor)

area, i.e. the blood glucose area below baseline, was significantly greater when glipizide was given before than when given together with the meal ($p < 0.05$).

Discussion

In healthy volunteers, the peak concentrations of glipizide showed a range of 346–988 nmol/l; the range of the time to reach peak concentration was 1.5–2.5 h in the fasting state and 1.5–4 h in the non-fasting state, and the AUC variation was about 3-fold. The most pronounced variation was seen in the elimination half-life, from 2.5 to more than 6 h. Previous studies, based on measurement of radiolabelled glipizide, have shown a half-life of about 2 h, but a figure of 6 h has also been presented [22]. Ongoing studies in patients indicate that the variation in elimination half-life may be even greater. As samples were not obtained beyond 2–3 h in the patients in the present study, no half-lives were calculated for these subjects. The patients displayed great variation in absorption.

Food intake in the volunteers, did not measurably influence peak concentrations, elimination half-lives or the AUC values; however, food intake prolonged the time to reach peak concentration by approximately 40 min. Data *in vitro* [23] indicate that glipizide dissolves poorly in simulated gastric fluid, but it dissolves very rapidly in simulated enteric fluid. Accordingly, it is reasonable to assume that food intake delays glipizide absorption by reducing the rate of gastric emptying.

As verified by the present findings, a single dose of glipizide 5 mg is capable of promoting a considerable increase in insulin release. In order to evoke optimal insulin release, however, the plasma concentration of glipizide should already have reached an effective level when the meal enters the gastrointestinal tract. This should suffice as a rationale for administration of glipizide in due time before rather than together with breakfast. The recorded, food-induced delay in glipizide absorption provides an additional argument in favour of this mode of administration. This is further emphasized by the findings in the patients. Assuming that the effective level is 100–200 nmol/l, this was reached within 5–10 min after the start of breakfast when the drug had been given 0.5 h before the meal, but similar levels were not reached until 30–45 min after the start of breakfast when the drug was given together with the meal. As previously reported [17], the preprandial administration was associated with earlier appearance of insulin and an improved disposition of blood glucose (data republished in the present report).

In conclusion, concurrent intake of food may delay the absorption of glipizide. This helps to explain why administration of glipizide 0.5 h before breakfast promotes a more appropriate relation between the plasma concentration of glipizide and the metabolic impact of the meal, thus yielding more optimal insulin release and better disposition of glucose than after concurrent intake of drug and breakfast.

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Impaired Effect of Sulfonylurea Following Increased Dosage*

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Summary. Ten Type 2 diabetics were examined during long-term treatment, at two dosage levels, with chlorpropamide once daily and glipizide t.i.d. Drug concentrations were measured by gas chromatography and high-pressure liquid chromatography, respectively, plasma insulin (IRI) by radio-immunoassay, and blood glucose enzymatically. Both drugs gave continuous sulfonylurea exposure, even at the lower dosage, and the mean plasma concentrations were almost doubled after the increase in dose. Neither the IRI nor the glucose response to meals showed any therapeutic improvement following the increase in chlorpropamide dosage. The lower dosage of glipizide produced better glucose utilization than chlorpropamide. On the other hand, the increased dose of glipizide led to impairment instead of further improvement. As this was associated with enhanced rather than reduced IRI levels, the impairment might have been due to increased peripheral insulin resistance. Thus, glipizide offers a therapeutic advantage over chlorpropamide, but its effectiveness may be restricted not only by limitations set by the disease, but also by counter-regulatory mechanisms that develop during continuous exposure to sulfonylureas at high levels.

Key words: diabetics, chlorpropamide, glipizide; dosage increase, impaired effect, blood glucose, plasma insulin

It is clinical experience that long-term sulfonylurea treatment is often insufficient. This may be due to a true inefficiency of sulfonylureas, but inadequate dosage may contribute to the therapeutic failure.

Previous studies have shown very great interindividual variation in the steady state concentrations of sulfonylureas. Indeed, some patients have had unde-

tectable or very low plasma concentrations of sulfonylureas, which may account for certain therapeutic failures [1–3]. There are also pronounced potency differences between sulfonylureas of the first (e.g. tolbutamide and chlorpropamide) and the second (e.g. glibenclamide and glipizide) generations [4]. In addition, at least glibenclamide and glipizide may improve glucose utilization not only by stimulating insulin release but also by enhancing the effects and utilization of insulin [5–7].

In order to study how changes in dosage might influence the therapeutic effects of a first- and a second-generation sulfonylurea, ten Type 2 diabetics were examined during long-term treatment with chlorpropamide and glipizide at two different dosage levels, with monitoring of the plasma concentrations of the drugs.

Material and Methods

Patients

One female and 9 male Type 2 diabetics, who were regular attenders at the Lund University Hospital, were studied after they had given informed consent to the investigation. Their ages were 47–66 years (mean $\pm$ SD 58 ± 6 years) and their mean body mass index (kg/m^2) was 28.0 ± 4.9 (range 18.7–34.3). All had normal hepatic and renal function tests. None had any endocrine disease apart from diabetes. All patients were seen by the same physician and had been found to respond unequivocally to dietary regulation and sulfonylurea.

Dosage of Chlorpropamide and Glipizide

For at least one month, 5 of the patients were initially kept on chlorpropamide¹, while the other 5 were taking glipizide². The former 5 were then switched to

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¹ Diabinese(e)®, Pfizer Corp., Groton, Conn., USA

² Min(i)diab®, Farmitalia Carlo Erba, Milano, Italy

glipizide, and the latter 5 to chlorpropamide. After at least another month on the second treatment, its dosage was increased (see further below). After at least one month on the third treatment, the patients on high dose chlorpropamide were switched to high dose glipizide, and vice versa.

The resulting mean daily doses were chlorpropamide 278 and 431 mg and glipizide 14.75 (5.25 + 4.75 + 4.75) and 25 (8.5 + 8.0 + 8.5) mg, respectively.

Intake of Drug and Meals on Examination Days

Each subject came in the fasting state to the Out-Patient Department of the Medical Clinic on four different occasions, after at least one month on each therapeutic regimen. An initial blood sample was taken at 8.00 a.m. A standardized breakfast was served at 8.30 a.m., a standardized lunch at 11.30 a.m., and a standardized dinner at 4.30 p.m. Both drugs were ingested at breakfast, and glipizide was also taken at lunch and dinner time.

Meal Composition

The breakfast consisted of low-fat milk (300 g), 2 slices of white bread (50 g) with butter (10 g) and cheese (35 g), and non-sweetened black coffee (150 ml). This yielded a total energy of 1800 kJ (430 kcal). The lunch comprised fried fillet of veal (100 g) with gravy, potatoes (150 g) and peas (70 g), canned peaches (150 g) and orange juice (200 g), yielding 2400 kJ (575 kcal). The dinner consisted of boiled hen (100 g) with parsley, gravy, potatoes (100 g), carrots (70 g), 1 slice of dark bread (25 g), butter (5 g) and low-fat milk (200 g), totalling 2150 kJ (510 kcal). Thus, the total energy intake per examination day was 6350 kJ (1515 kcal), as 22% protein, 33% fat and 45% carbohydrate.

Blood Sampling

Serial blood samples were taken through an indwelling antebrachial venous polyethylene catheter. On each occasion, 1–2 ml blood were drawn and discarded before the 10 ml samples were collected. Samples for subsequent determination of blood glucose, and plasma insulin, chlorpropamide and glipizide were collected –30, 0, 30, 60, 120, 180, 210, 240, 300, 360, 420, 480, 510, 540 and 600 min after breakfast.

Analyses

The concentration of blood glucose was determined by the hexokinase method [8] and that of plasma immunoreactive insulin (IRI) by a solid phase radioim-

munoassay (Phadebas Insulin Test®, Pharmacia, Uppsala, Sweden) [9]. The plasma concentration of chlorpropamide was determined by gas chromatography [10], and that of glipizide by high-pressure liquid chromatography [11].

Statistical Analysis

The statistical significance of differences between paired data was calculated by Wilcoxon's signed-rank test.

Results

Plasma Concentrations of Chlorpropamide and Glipizide

In every patient, the concentration of chlorpropamide was significantly ($p < 0.01$) higher at each interval during treatment with the larger than with the smaller dose (Fig. 1, middle panel). The situation was similar for glipizide, the difference reaching significance at 0 ($p < 0.01$), 60 ($p < 0.05$), 120, 180 ($p < 0.01$), 360, 420 ($p < 0.05$), 480 ($p < 0.01$) and 510 ($p < 0.05$) min (Fig. 2, middle panel). One patient had no detectable chlorpropamide in plasma collected before dosing during the low dosage regimen (250 mg), and another patient had an undetectable concentration of glipizide prior to the morning dose during low dose treatment (5 mg t.i.d.). The pre-dose concentrations in these patients became measurable after the dose was increased to 375 mg once daily and 10 mg t.i.d., respectively. All other patients had detectable pre-morning dose concentrations of chlorpropamide and glipizide, during both the lower and the higher dosage regimens. For both drugs the mean plasma concentration was almost doubled following the increase in dose (Figs. 1 and 2).

Plasma Immunoreactive Insulin (IRI)

The plasma IRI response to breakfast, lunch and dinner at the two chlorpropamide dose levels are shown in Fig. 1 (lower panel). The mean fasting IRI levels were almost identical: 19 ± 4 ($\bar{m} \pm \text{SEM}$; low dosage) and 18 ± 4 (high dosage) mIU/l, respectively. There was no consistent difference in plasma IRI response between the two dosage regimens.

The plasma IRI response to breakfast, lunch and dinner at the two glipizide dose levels are illustrated in Fig. 2 (lower panel). The mean fasting IRI levels were identical: 19 ± 4 (low dosage) and 19 ± 5 (high dosage) mIU/ml, respectively, and were very similar to those seen during chlorpropamide treatment (see

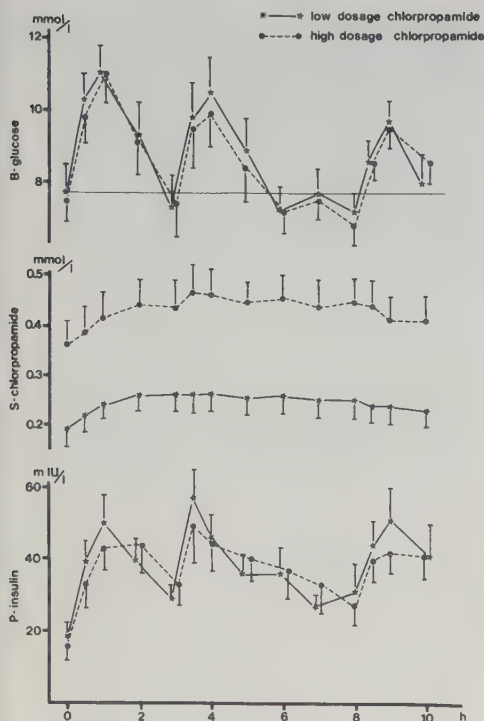


Fig. 1. Mean ($\pm$ SEM) concentrations of blood glucose (upper panel), plasma immunoreactive insulin (IRI) (lower panel) and plasma chlorpropamide (middle panel) in 10 Type 2 diabetics before and after intake of a standardized breakfast, lunch and dinner during long-term treatment with chlorpropamide in a mean dose of 278 mg/day ($\star$ — $\star$) and 431 mg/day ($\bullet$ — $\bullet$). The difference between the chlorpropamide levels was significant ($p < 0.01$) at each interval recorded, but no significant difference was recorded for plasma IRI or blood glucose. Fasting blood glucose at lower dosage level indicated by thin line

above). However, the responses to meals seemed different. At most intervals, the plasma IRI concentrations were numerically higher during treatment with the higher than with the lower dose, although only the difference at 360 min reached significance ($p < 0.05$). Also, the area above the fasting level was numerically, albeit not significantly, greater.

Blood Glucose

The blood glucose changes during the chlorpropamide treatments are shown in Fig. 1 (upper panel),

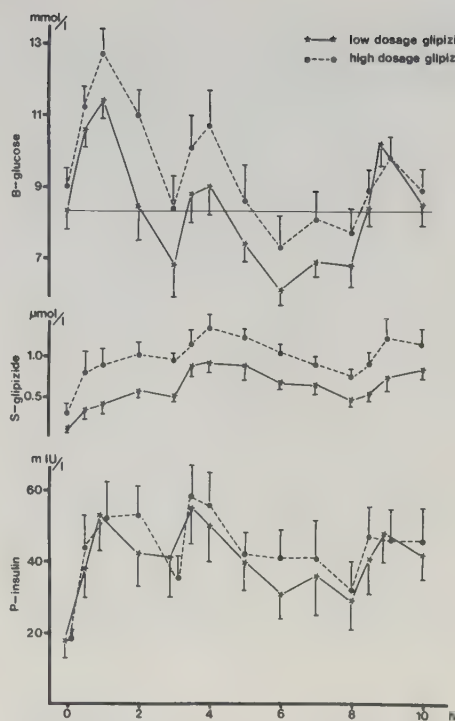


Fig. 2. Mean ($\pm$ SEM) concentrations of blood glucose (upper panel), plasma immunoreactive insulin (IRI) (lower panel) and plasma glipizide (middle panel) in 10 Type 2 diabetics before and after intake of a standardized breakfast, lunch and dinner during long-term treatment with glipizide t.i.d. in a mean daily dose of 14.75 mg/day ($\star$ — $\star$) and 25 mg/day ($\bullet$ — $\bullet$). The difference between the glipizide levels was initially significant (before morning dose, $p < 0.01$) and at 120, 180 ($p < 0.01$), 240, 420 ($p < 0.05$) and 450 ($p < 0.01$) min. The blood glucose level (area) following the higher dosage was significantly greater ($p < 0.01$) than during the lower dosage. Fasting blood glucose level at lower dosage indicated by thin line

and those during the glipizide treatments in Fig. 2 (upper panel). The mean ($\pm$ SEM) fasting levels were not significantly different from each other: 7.7 ± 0.8 mmol/l (low dose chlorpropamide), 7.5 ± 0.6 (high dose chlorpropamide), 8.3 ± 0.5 (low dose glipizide) and 9.0 ± 0.5 (high dose glipizide) mmol/l.

During the two chlorpropamide dosage regimens, the blood glucose responses to meals were virtually identical, and the glucose concentrations almost invariably remained above the fasting level. In contrast,

Table 1. Body weights (kg) of patients at the end of the treatment periods

Patient no.	Chlorpropamide		Glipizide	
	Low dosage	High dosage	Low dosage	High dosage
1	86	88	84.5	86.5
2	82	83.5	83	82
3	116	121.5	120	123
4	78	80	78	79.5
5	101.5	99	100.5	103
6	76	74.5	76	76
7	98	98	102	101
8	45	47	46	46.5
9	68.5	70.5	71	70
10	65	64.5	64.5	65
	N.S.*		N.S.*	
$\bar{X}$	81.6	82.7	82.5	83.4
SD	20.3	20.7	21.0	21.7

* in intraindividual Wilcoxon test

the low dose of glipizide promoted a considerable reduction in blood glucose below the fasting level, the concentrations being significantly lower than those during chlorpropamide treatment ($p < 0.01$ for net areas; i. e. area above minus area below fasting level). On the other hand, the increased dose of glipizide was accompanied by an increase instead of a further fall in blood glucose levels ($p < 0.01$ vs low dosage).

Body Weight

There was no significant increase in body weight following high-dose treatment with either of the drugs (Table 1).

Discussion

As the entire study period covered several months, it could be argued that the therapeutic results were not comparable, because of progression of the disease or change in body weight. This is not very likely, however, because neither the fasting IRI nor the fasting blood glucose level differed significantly between treatments, and there was no significant alteration in body weight. Hence, it seems justified to analyze the different responses to meals as due to differences in the drug treatments. Additional justification for this conclusion is given below.

The drug level measurements demonstrated that the increases in dosage had enhanced the exposure to sulfonylurea, the mean concentrations being almost doubled. In addition, it was seen that, with one exception for each drug, all patients were continuously exposed to sulfonylurea already at the lower dosage. As

the exceptional cases, too, became continuously exposed after the increase in dose, there is no particular reason to suspect that those subjects had complied less well with their medication than the others.

In spite of the marked elevation in plasma chlorpropamide concentration after the increase in dosage, neither the plasma IRI nor the blood glucose responses showed any therapeutic improvement. Accordingly, the maximum effect of chlorpropamide appears to have been reached at the lower dosage, or perhaps the concentration – effect curve for chlorpropamide is so flat that doubling the drug concentration would not suffice to enhance its effect.

The low dosage of glipizide promoted a better therapeutic effect than either dose of chlorpropamide, as judged from the considerable improvement in blood glucose levels. This emphasizes that second-generation sulfonylureas, such as glipizide and glibenclamide, are more potent antidiabetic agents than are those of the first generation [cf. 4]. Moreover, as there was no corresponding increase in IRI levels, the improved glucose utilization probably resulted from an extrapancreatic effect, e. g. an increased number of insulin receptors [5, 6].

Despite the significant increase in plasma glipizide, the increase in the dose of glipizide did not further improve its therapeutic effect. On the contrary, the blood glucose concentrations were significantly elevated, sometimes even exceeding the levels recorded during chlorpropamide treatment. Such impairment was seen both in those who were on high-dosage glipizide beforehand and in those who took it after chlorpropamide. Hence, the impaired response could hardly have been due to progression of the disease, but was rather a consequence of treatment. Furthermore, the impairment could not be explained by reduced production of insulin [cf. 12, 13], as the IRI levels were higher rather than lower after the dosage increase. Accordingly, the impaired glucose utilization seen during high-dosage glipizide would seem consequent to either an influence on the secretion of hormones other than insulin, e. g. glucagon, or to an extrapancreatic effect.

As emphasized above, glipizide and glibenclamide can increase the number of insulin receptors, and this would help to promote reduction in a blood glucose [5, 6]. However, it is also known that chronically high levels of circulating insulin have the opposite effect, leading to increased insulin resistance [14, 15]. It follows that during chronic exposure to high glipizide concentrations, the stimulant effect on insulin receptors would be counteracted by the chronic elevation of circulating insulin. As the IRI levels were higher rather than lower during high-dosage as compared to low-dosage glipizide, the impaired glucose

utilization could be an expression of increased insulin resistance, subsequent to hypersecretion of insulin.

In conclusion, the therapeutic effect of sulfonylureas may be restricted not only by limitations set by the disease, but also by counterregulatory mechanisms that develop during continuous exposure to high levels of sulfonylurea.

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THERAPEUTIC EQUIVALENCE OF ONCE- AND THRICE-DAILY GLIPIZIDE

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Key words: type 2 diabetes, glipizide, once vs. thrice daily

Summary

Two cross-over studies were carried out in 23 patients with type 2 diabetes, to examine whether glipizide, a potent sulfonylurea with fast and complete absorption and rapid elimination ($t_{1/2} < 5$ h), can be given once-daily without loss of therapeutic effect. In both studies, patients were randomly assigned to an initial dosage of 7.5 mg once daily or 2.5 mg thrice daily, and the dosage was increased to 15 mg o.d. or 5 mg t.i.d. if fasting plasma glucose remained over 10 mmol/l on the lower dosage. In study 1 (n=11), administration once-daily before breakfast was compared with intake before breakfast, lunch and early dinner (5 p.m.) while study 2 (n=12) compared intake once-daily before breakfast with intake before breakfast, lunch, and at bedtime (10 p.m.).

Neither the 24-hour urinary glucose excretion nor HbA_{1c}, fasting plasma glucose, insulin or C-peptide levels differed between once-daily and thrice-daily administration with the third dose given before early dinner. The nadir plasma levels of glipizide were not significantly different and often too low to be detected. Postponing the third dose until 10 p.m. did not evoke any improvement in HbA_{1c} or in fasting plasma glucose, insulin or C-peptide. The mean nadir glipizide levels following this schedule were twice as high as following once-daily administration.

As expected, the after-breakfast plasma levels of glipizide were higher when the whole dose was taken before breakfast than when dosage was divided. The corresponding plasma levels of insulin were higher, and those of plasma glucose lower. The after-lunch levels of glipizide did not differ significantly, and there was no after-lunch difference with respect to plasma insulin and glucose.

It appears that the major effect of glipizide is to augment insulin availability following meals, whilst it has little influence on nocturnal glucose control. At a daily dose of 7.5 mg or more, glipizide can be taken once daily without loss of efficacy, at least in areas with Northern European meal schedules.

Glipizide* is a potent sulfonylurea with fast absorption and complete

*Min(i)diab, Farmitalia Carlo Erba, Milano, Italy; Glucotrol, Roerig/Pfizer, New York, N.Y.

bioavailability (1). It is rapidly eliminated with a plasma half-life of 2-5 h (1, 2), which could justify administration in three or more daily doses. As the efficacy of glipizide is increased when it is taken half an hour before breakfast (3), it is common practice to administer glipizide before the three major meals. On the other hand it has been reported that, at a daily dosage of 7.5-20 mg, the degree of glucose control was similar when the drug was taken once daily before breakfast and when taken before breakfast, lunch and dinner (4). However, as the third dose was given before an early (5 p.m.) dinner, it is possible that later administration of the third dose would improve nocturnal and fasting glucose levels. To examine this possibility, treatment by administration once daily was compared with that of two different thrice daily dosage schedules, before early (5 p.m.) dinner and at bedtime (10 p.m.).

SUBJECTS AND METHODS

Trial design

Both studies were carried out as randomized, open, ambulatory cross-over trials. All patients had previously been on medication with oral antidiabetic drugs which in each case were withdrawn one week before glipizide treatment was commenced. Every patient received personal dietary instructions, to be maintained during all phases of the study. The patients were informed about the nature, purpose and possible risks of the study before their consent to participate was obtained. The study protocol was approved by the Committee on Ethics of the Helsinki Central University Hospital.

Study 1

Eleven patients (5 females and 6 males) with type 2 (non-insulin dependent) diabetes mellitus (NIDDM, type 2) were recruited at the Helsinki University Central Hospital. Their mean age $\pm$ SEM was 54 ± 2 years (range 41-62), mean duration of diabetes 6 ± 1 years (range 2-16) and mean relative body weight $119 \pm 3\%$ (range 104-135). They had no signs of diabetic complications, no history of recent myocardial infarction, nor clinical evidence of cardiac, pulmonary, hepatic or renal dysfunction. Three patients were treated with antihypertensive drugs, and this treatment was kept

unaltered throughout the study.

The patients were randomly allocated to treatment with glipizide 2.5 mg t.i.d. or 7.5 mg o.d. If fasting plasma glucose after 2 weeks exceeded 10 mmol/l, the dosage was increased to 5 mg t.i.d. or 15 mg o.d., respectively. After an 8-week period on the final dosage, the patients switched over to the alternative dosage schedule for an equal time period. Nine patients remained on the initial 7.5 mg daily dosage while an increase to 15 mg was made in two cases. When glipizide was given once daily, it was administered 1/2 h before breakfast. When given thrice daily, it was administered 1/2 h before 8 a.m. breakfast, 11.30 a.m. lunch and 5 p.m. dinner.

Body weight, fasting plasma glucose, 24-hour urinary glucose excretion (dU-glucose) and glycosylated hemoglobin (HbA_{1c}) were measured before and after each treatment period. The pre-dose (trough) serum concentrations of glipizide were determined in the morning (7.30 a.m.) during each period.

At the end of each treatment period, concentration profiles of plasma glucose, serum insulin (IRI), serum C-peptide and serum glipizide were obtained. Samples were taken at 8.00, 8.15, 8.30, 8.45, 9.00, 10.00, 11.00, 12.30 a.m., and at 1.30 and 3.00 p.m. At 8.00 a.m. the patients received a standard breakfast of 133 g Complian^(R) and 125 ml orange juice, signifying 625 kcal (84 g carbohydrate, 21 g fat and 27 g protein), at 11.30 a.m. a standardized lunch of 590 kcal (68 g carbohydrate, 22 g fat and 24 g protein), and at 2.00 p.m. a snack consisting of coffee, 125 ml orange juice and biscuits, corresponding to 75 kcal (16 g carbohydrate, 2 g fat and 2 g protein).

Study 2

Twelve other NIDDM patients (6 females and 6 males) were recruited at the Seinäjoki Central Hospital. Their mean age $\pm$ SEM was 65 ± 2 years (range 55-71), mean duration of diabetes 7 ± 1 years (range 1-15) and mean relative body weight $120 \pm 5\%$ (range 96-155). Like the patients in study 1, none had any signs of diabetic complications, any history of recent myocardial infarction or clinical evidence of cardiac, pulmonary, hepatic or renal dysfunction. Five patients were treated with either thiazides or digitalis, or a combination thereof. One of these patients was also given methyldopa, another metoprolol, and a third one clonidine. All these medications were kept unaltered throughout the study.

As in study 1, the patients were randomly allocated to treatment with glipizide 2.5 mg t.i.d. or 7.5 mg o.d. and, if fasting plasma glucose after 2 weeks exceeded 10 mmol/l, the dosage was increased to 5 mg t.i.d. and 15 mg o.d., respectively. After a 4-week period on the final dosage, the patients switched over to the alternative dosage schedule. Six patients remained on the initial 7.5 mg daily dosage while an increase to 15 mg was made in the other 6 cases. When glipizide was given once daily, it was administered 1/2 h before breakfast. When given thrice daily, it was administered 1/2 h before breakfast and lunch and at bedtime, 10 p.m. Dinner time was usually 5 p.m.

Body weight, fasting plasma glucose, fC-peptide, insulin and HbA_{1c} were measured before and after each treatment period.

Assays

Plasma glucose was determined by a glucose oxidase method, urine glucose by polarimetry, and HbA_{1c} by micro-column chromatography (Iso-lab). Serum insulin and C-peptide were measured by radioimmunoassays (5, 6). The serum concentrations of glipizide were determined by high-pressure liquid chromatography (7).

Statistics

The significance of differences between treatment periods was assessed by Wilcoxon tests on intraindividual data pairs. Means are given $\pm$ SEM.

RESULTS

Once daily vs. thrice daily dosage with third dose given before 5 p.m. dinner (Study 1)

The mean body weight (kg $\pm$ SEM) of the patients did not differ between treatment schedules (74.2 $\pm$ 2.7 vs 74.8 $\pm$ 2.7), and there were no between-schedule differences in 24-hour urinary excretion of glucose, HbA_{1c}, fasting plasma glucose, insulin or C-peptide (Table I). During both schedules 5 patients had undetectable nadir concentrations of glipizide. The mean nadir level of glipizide was numerically but not significantly lower during once-daily than during t.i.d. treatment (Table I).

As expected, the after-breakfast drug levels ($AUC_{8-11 \text{ a.m.}}$) were much higher ($p < 0.01$) when the whole daily dose had been taken before breakfast (Fig. 1a). After lunch (11 a.m.-3 p.m.), the AUC values did not differ significantly (Fig. 1a).

Analogously, the after-breakfast plasma glucose increase ($\Delta AUC_{8-11 \text{ a.m.}}$), but not that after lunch ($\Delta AUC_{11 \text{ a.m.-3 p.m.}}$), was lower ($p < 0.05$) during once-daily than during thrice-daily dosage (Fig. 1 b).

Similarly, the after-breakfast but not the after-lunch levels ($AUC_{8-11 \text{ a.m.}}$) of insulin were higher ($p < 0.01$) during the once-daily regimen (Fig. 1c). The corresponding C-peptide levels were numerically but not significantly higher (Fig. 1c).

Once-daily vs. thrice-daily dosage with third dose given at 10 p.m. (Study 2)

When the third dose was given at bedtime, the nadir levels of glipizide were twice as high as during once daily treatment (Table I). This difference did not reach statistical significance, because of the pronounced variation. As further shown in Table I, there were no between-schedule differences in HbA_1 or fasting plasma glucose, insulin or C-peptide.

DISCUSSION

After intake of 2.5-5 mg glipizide, effective plasma concentrations ($> 50-100$ ng/ml) may prevail for 6-12 h (1). Therefore, a dose of 7.5 mg or more should be able to maintain an effective drug level in plasma for a large part of a 24-hour period. In accordance herewith, Östman *et al.* (4) found that once daily glipizide, 7.5-20 mg before breakfast, was as effective on glucose and insulin levels as divided dosage with intake before breakfast, lunch and early (5 p.m.) dinner.

In further support, neither the 24-hour excretion of glucose nor HbA_1 , fasting plasma glucose, insulin or C-peptide differed between o.d. and t.i.d. (third dose before 5 p.m. dinner) treatments in the present study 1. In addition, once-daily administration improved glucose control after breakfast, when blood glucose elevation is most prominent, and it was at least as effective

on after-lunch glucose as the t.i.d. regimen. The after-dinner glucose excursions were not measured, but they can hardly have been relevantly higher during once-daily medication, as the 24-hour urinary glucose excretion was lower rather than higher during this regimen. Accordingly, it seems confirmed that once-daily dosage of glipizide is at least as effective as t.i.d. dosage with the third dose given before early dinner. The o.d. regimen also offers a practical advantage, liable to improve compliance with therapy.

In view of the short elimination half-life of glipizide (< 5 h), administration of the third dose as early as 4.30 p.m. could lead to ineffective nocturnal levels of the drug, and several patients had undetectable pre-dose concentrations of glipizide during this therapeutic regimen. However, study 2 showed that postponing the third dose intake until 10 p.m., which should maintain effective glipizide levels at least until 4 a.m., and in fact did so even until 7.30 a.m. did not improve the fasting concentrations of any parameter measured. This indicates that glipizide is relatively ineffective on the regulation of nocturnal, i.e. fasting, glucose, and it emphasizes that the major effect of glipizide is to enhance the availability of insulin following meals. It is of interest in this context that glipizide may undergo enterohepatic recirculation, allowing the drug to be reabsorbed during meals (2); this could help to explain why once-daily glipizide can be as effective as t.i.d. administration.

The present studies were based upon Northern European meal schedules with early lunch and dinner, and it is possible that a comparison between once-daily administration and t.i.d. treatment with the third dose given before a late (Southern European) dinner could have given a different result. Therefore, the practical once-daily dosage schedule of glipizide (7.5 mg or more) can be recommended only in areas with Northern European meal schedules.

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Table 1. Therapeutic control in 11 patients (1) + 12 (2) with type 2 diabetes during treatment with glipizide once daily before breakfast and three times daily, either before breakfast, lunch and 5 p.m. dinner (1), or with the third dose given at 10 p.m. (2). No between-schedule differences were significant.

	Study 1		Study 2	
	Once daily	Thrice daily	Once daily	Thrice daily
Fasting plasma glucose (mmol/l)	8.8 ± 0.6	9.2 ± 0.7	10.9 ± 0.7	10.9 ± 0.8
24-h urine glucose (g/24 h)	9 ± 4	17 ± 7	*	*
Hemoglobin A ₁ (%)	9.9 ± 0.4	10.0 ± 0.4	10.1 ± 0.5	9.9 ± 0.5
Fasting serum insulin (nmol/l)	0.12 ± 0.02	0.13 ± 0.03	0.20 ± 0.03	0.24 ± 0.04
Fasting serum C-peptide (nmol/l)	0.66 ± 0.08	0.66 ± 0.07	0.49 ± 0.06	0.52 ± 0.08
Fasting (nadir) serum glipizide (ng/ml)	26 ± 13**	42 ± 18**	70 ± 21***	140 ± 33***

* not measured

** 9 patients on 7.5 and 2 patients on 15 mg daily

*** 6 patients on 7.5 and 6 patients on 15 mg daily

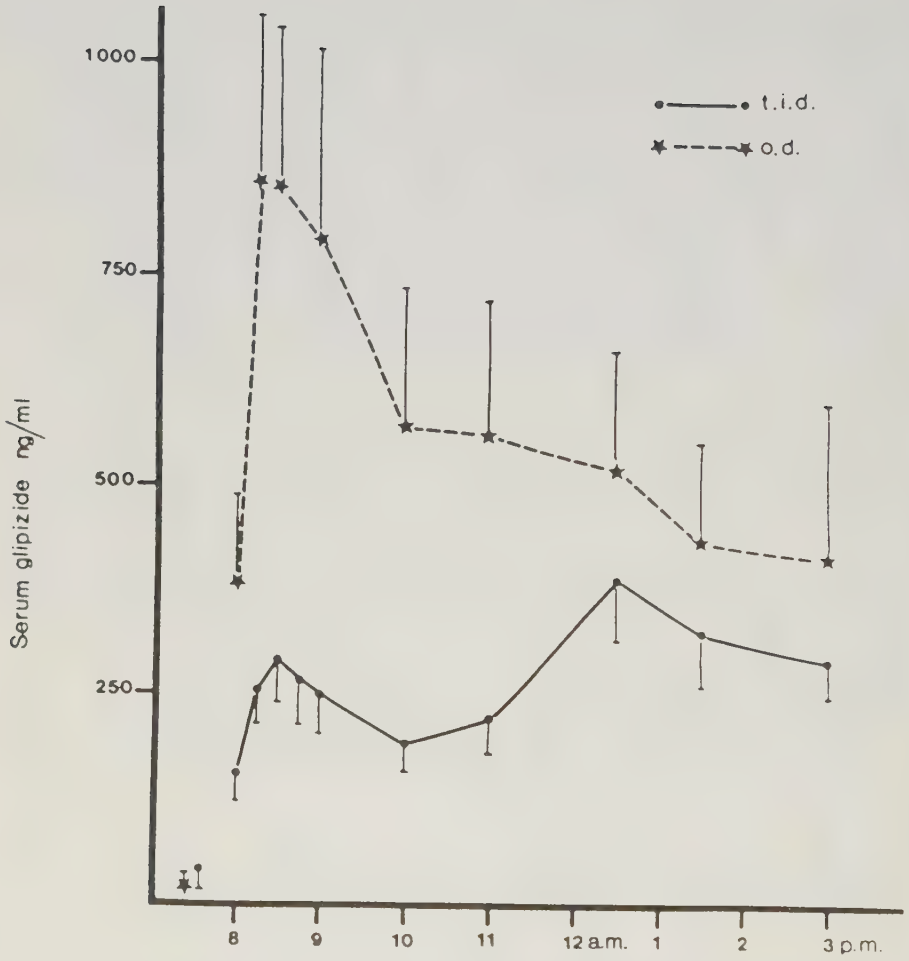


Fig. 1 a

Time-concentration curves of serum glipizide in 11 patients with type 2 diabetes during treatment with glipizide once daily (o.d.) and thrice daily (t.i.d.), with the third dose given before early (5 p.m.) dinner. Means $\pm$ SEM indicated.

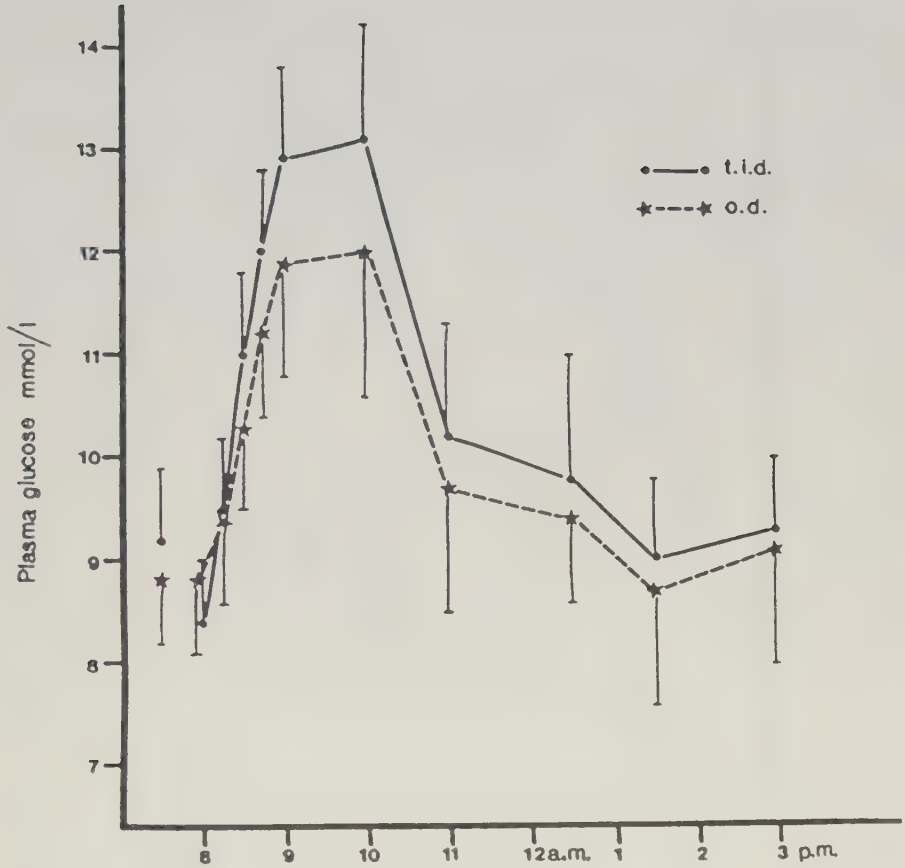


Fig. 1 b

Time-concentration curves of plasma glucose in 11 patients with type 2 diabetes during treatment with glipizide once daily (o.d.) and thrice daily (t.i.d.), with the third dose given before early (5 p.m.) dinner. Means $\pm$ SEM indicated.

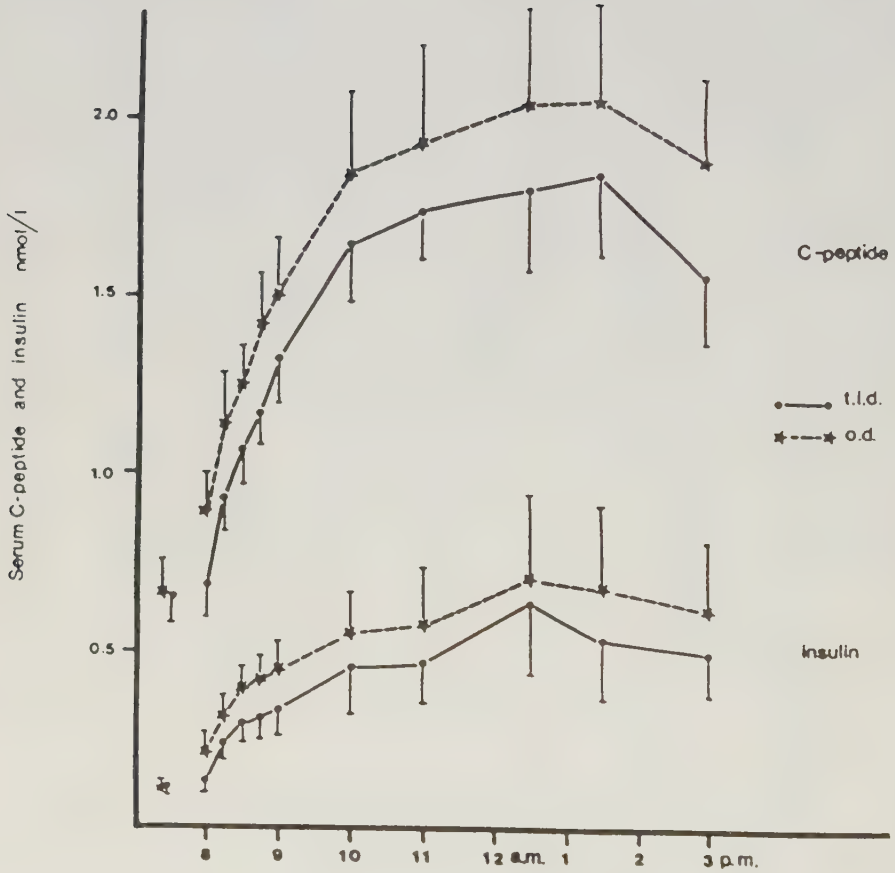


Fig. 1 c

Time-concentration curves of serum insulin and serum C-peptide in 11 patients with type 2 diabetes during treatment with glipizide once daily (o.d.) and thrice daily (t.i.d.), with the third dose given before early (5 p.m.) dinner. Means $\pm$ SEM indicated.

